

Next slide please

“ I THOUGHT that in the eight minutes I’ve got I’d bring you up to date on what our group has been doing in the last year; in a sense this is a progress report and updates the paper we gave here last year; I won’t go over the nomenclature again; could I have the first slide please—oh, I think you must have someone else’s box—mine is the grey one with my name on the top, no, wait a minute, not my name, whose name was it now? ah yes, you’ve found it; there’s a red spot on the top right hand side of each slide that is the side that becomes the bottom left when you project it, OK, you’ve got it now, let’s have a look, no, that’s the last slide not the first, yes, now you’ve got the right one but it’s on its side, what about the red dot? there are two? well anyway turn it through ninety degrees, no, the other way, yes now we’re there, perhaps we could have the lights off, well I’m sorry there are probably too many words on this slide, and the printing is a bit thin; can you read it at the back? you can’t; well I’d better read it out; no I won’t, it’s all in the paper which should be published within a month or so, and anyone who wants I’ll give a preprint to afterwards, anyway, for those who can read it, this slide is a block diagram of the purification process we used and before I go any further I should mention that there are a couple of misprints: on the third row, fourth box from the left, well, of course that’s the second box from the right, if you can read it, it says alkaline, now that should be acidic; also you can perhaps see the word mebmrane, that should of course be membrane; now if I can have a look at the next slide—now which one is this? ah, yes it’s the scatter diagram, I haven’t marked the quantities but we are plotting concentration against particle size; if I remember rightly this has been normalised; perhaps I could have the lights for a moment to check in the text, yes, here we are, well it doesn’t actually say—we could work it out but it’s probably not worth the time, so if I could have the lights off, let’s have a look at the plot; well I think you can see a sort of linear relationship—

there’s a fair bit of scatter, of course, but I think the data are at least suggestive; perhaps if I held up a pointer you could see the relationship more clearly—I expect there’s a pointer around somewhere, no I won’t need the lights, yes here it is, now you can see the trend and there’s just the hint of another trend running sub-parallel to it through this other cluster of points, you may see that more clearly if I slide the pointer across to the other—no, I wasn’t saying next slide, just that I would slide the pointer; anyway now the next slide is up let’s keep it on the screen, now this is the sort of evidence on which the data in the last slide were based; this is a thin section—it could take just a bit of focusing—yes, that’s better, it’s difficult to get the whole slide in focus at once, now the scale is, well that bar is one micron long, hang on what am I saying? it’s ten microns long—oh dear, the chairman is giving me the two minute warning, it’s difficult to give you a clear picture of this work in only eight minutes, but let’s plough on, what was I saying? ah yes, that bar is ten microns long, now if we turn to the next slide, please, this is the result of a chemical analysis of the dark region that is near the centre of that thin section, is it possible to go back a slide? well not to worry, you can see in the analysis how dominant—sorry what was that? oh yes, the errors are plus or minus a per cent or so—that’s the standard deviation, no it can’t be, it must be the standard error of the mean—oh dear, the chairman says my time is up, can I beg half a minute—are there any more slides? really? well let’s skip the next two, now this one is pretty important, it brings together several of the threads that you’ve probably been able to discern running through this talk, but rather than go through it in detail perhaps I should have the lights and just put up one or two key numbers on the blackboard—the chairman says there’s no chalk, well it’s all in the paper I was mentioning anyway perhaps I’ve been able to give you the gist of what we’ve been doing, I guess that’s all I’ve got time for.”



Working out a UK energy policy

The UK government's consultative document on energy policy was published last February. Frank Hooley, a Labour MP, criticises some of its assumptions and suggests ways of modifying it.

THERE is no such thing as an 'energy gap'. This mischievous and misleading notion has been carefully fostered, though maybe not invented, by the nuclear power interests to scare people into accepting ambitious and unrealistic nuclear power programmes, which perforce are being constantly scaled down even by their own inventors.

Vast quantities of energy stream down every day from the sun, and will continue to do so as long as the earth is part of the solar system. Immense amounts of energy reside in the waves of the sea, in the tides, in the wind, and in the natural heat of the earth itself. Since energy from these sources exists in quantities far beyond the needs of mankind and since it is *infinitely renewable*, it is illogical to talk of an 'energy gap'.

But there is a 'technology gap'. We cannot at present harness the sun, wind, tides and waves on a scale sufficient to meet all our energy needs. Nevertheless it must be a central objective of a rational energy policy to switch steadily over to the use of renewable resources and rely more on the energy revenue of this planet than its energy capital of fossil fuels. It is a serious weakness of the Green Paper that it fails to grasp this principle.

Another myth is that there is a direct correlation between a higher material standard of living and greater consumption of energy. This is too simplistic.

The Green Paper quite fairly points out that our homes, which use roughly a quarter of the country's energy, are now supplied by only 6% more heat than they received in 1950, and that the amount of heat supplied per household has decreased since that date (the number of households has gone up by 2½ million). Yet it is beyond question that the general standard of heating and comfort in most homes today is higher than that enjoyed a generation ago. Moreover there is plenty of scope for this process to continue, since only a tiny fraction of our total housing stock has anything like adequate insulation as yet. In the domestic sector there has been, and may well continue to be, an inverse correlation between energy consumption and standard of living.

This process is not confined to the domestic sector. The Green Paper mentions that over the 25 years up to 1975 the energy used per tonne of crude steel produced declined at a rate of 2¼% per annum. Other industries may well show a similar pattern.

The above two examples, both taken from the Green Paper, are all the more striking because they cover a period, 1950–75 when oil in particular was extremely cheap (and becoming cheaper in real terms up to 1974), and virtually no efforts were being made in conservation.

Yet the Green Paper repeatedly extrapolates energy demand on the basis of certain given percentages of economic growth. What is worse, demands in the various sectors have been "estimated from structural relationships between the economic and other relevant variables based on the historic period up to 1975"—that is precisely the period when (until 1974) oil was dirt cheap and efforts at conservation non-existent! The ominous fact is that energy demand reached a peak in 1973 to which in half a decade it has not yet returned.

It is important to dwell on these two fallacies—the 'energy gap' and the idea of direct correlation between



The energy tripod: nuclear, conservation, coal

economic growth and increased energy consumption—because they seem to condition implicitly and explicitly the thinking in the Green Paper. In spite of the fact that growth of heat supplied to the domestic sector was only 6% over 25 years to 1975 the Green Paper assumes another 7% rise (¼% per annum compound) in the next 22 years. Similarly in respect of steel production technological advance is suddenly assumed to provide a saving of only ½% per tonne per annum from now to the year 2000.

The Green Paper policy

The long-term strategy favoured by the Green Paper is based on the tripod: coal; conservation; nuclear. The calculations about the life of oil and gas supplies from the North Sea seem to me to be unduly gloomy, and given stringent depletion policies and strong efforts in the field of conservation they could surely be made to carry us through the next 40 years. 3½ billion tonnes of oil reserves, the lower estimate, would give 35 years' supply at our current annual consumption of 100 million tonnes. The Gas Corporation is publicly predicting that it can maintain supplies for 50 years; given long-term contracts with Norway and the Middle East this may be an underestimate. Let us look at the Green Paper tripod.

Conservation It is impossible to dissent from this as a major policy objective. The allowance for conservation equivalent "to an overall reduction of over 23% in heat supplied by the end of the century" is an ambitious target. It seems to rely on home insulation, vehicle economy, industrial fuel efficiency. These are essential but not enough attention is paid to new fuel burning techniques such as fluidised bed combustion and combined heat and power systems. The latter could have enormous potential applied to industry.

Coal The importance of coal as our major indigenous fossil fuel resource is self-evident but the prospect of raising 170 million tonnes per annum by the year 2000 seems to me to be bleak. Production in the past six years has varied from 120 to 129 million tonnes (forgetting the 1974 disaster) and I can see no will to boost it 40% above that.

The Green Paper is right to suggest the importance of turning coal into substitute natural gas (SNG); by doing this about half the thermal value of the coal could be made use of, whereas when burnt in power stations now 70% is sheer waste. Coal is clearly the pivot of our energy strategy, but can we mine enough of it?

Nuclear This is the wobbly leg of the Green Paper tripod. First, it is fascinating to note the remarkable way in which nuclear ambitions have been scaled down. According to the report of the Royal Commission on Environmental

Pollution (Flowers) the Atomic Energy Authority was at one point calculating as desirable an installed nuclear generating capacity of 210–285 GW by the year 2000. This was then reduced to 149GW and further reduced to 104GW.

In the Green Paper the target has been cut again to a maximum total of 40GW installed nuclear capacity by the year 2000 which would include stations already built or being built. So within four years our nuclear aspirations have come down with a crash from 280 to 40GW capacity! How seriously can the layman be expected to take calculations which vary in this ludicrous fashion over so short a period of time. To be fair a similar sobering down of wild nuclear fantasies has occurred in Western Europe at large.

If we take away the 10GW we already have from the 40GW figure and assume 1.25GW capacity for each nuclear power station, 24 nuclear stations will have to be completed in the last 15 years of the century, since anything ordered now will take at least seven years to complete. The UK nuclear industry has never demonstrated the remotest ability to build 24 stations in 15 years. By 1985 the cost will be about £1,000 million a time, so we are thinking in terms of £24 billion expenditure for nuclear electricity alone!

Thirdly, we already have 37% generating capacity greater than maximum demand not counting 15GW of new plant under construction, and sales of electricity have been declining or static for the past five years.

The nuclear industry still cannot decide if it wants a gas-cooled or water-cooled system—after persisting with gas-cooled for 30 years, Magnox and AGR, the Department of Energy has now taken out an option on the PWR, so the argument about the two systems will go on interminably and duplication of effort will continue.

The great international debate over reprocessing, nuclear waste, nuclear accidents, terrorism, civil rights, proliferation of nuclear weapons, and all the problems of a 'plutonium economy' is only now beginning to get into its stride. It is causing doubts and fears in the United States, Sweden, France, Germany, Australia and has led to diplomatic arguments with countries as far apart as Brazil and Pakistan. In the UK the Flowers Report has been followed by the Windscale argument and that will be followed by another ferocious public debate about the first fast breeder reactor—so public concern is not just going to die away.

A modified energy strategy

There is no need in my view to reject wholesale the energy policy set forth in the Green Paper. In particular I would entirely support the stress laid on conservation and fuel efficiency and the importance of our coal reserves in our energy calculations.

I would seriously put forward the following suggestions for modifying the Green Paper policy:

- We should aim at a depletion policy and conservation effort which would ensure a minimum life of 40 years for our oil and natural gas resources. To achieve this we might need a judicious mix of imported fuel in the form of long-term contracts for oil and gas from the Norwegians, and for Liquefied Natural Gas (LNG) from North Africa and the Gulf. It is appalling that billions of cubic feet of natural gas associated with oil exploitation in the Gulf is being flared off to waste every day. We should offer our technology to stop this waste and make use of this precious irreplaceable source of energy. More effort should also be put into the production of SNG from our own coal.
- The disproportionate R & D effort on nuclear power should be drastically scaled down and the proposed extension of the Windscale reprocessing plant abandoned. The two AGRs already ordered will no doubt have to go ahead, but nothing should be ordered beyond that until there is an international consensus on matters such as the disposal of nuclear waste and the tolerable social and environmental

hazards of nuclear power. There should be no work on a Commercial Fast Breeder Reactor. The top limit of the nuclear contribution by 2000 should be about 15GW.

- Considerable effort should be devoted to the development of combined heat and power systems—especially for industrial use—and investment capital made available through the National Enterprise Board to firms who could manufacture the equipment needed.

- Fluidised bed combustion for higher efficiency of coal burning needs much more vigorous development.

- Within the R & D budget of the public sector there should be a switch away from nuclear towards renewable energy sources. The present allocation of about 60% nuclear and 40% for all the rest is a gross distortion and there should be a massive and sustained expansion of R & D effort in the field of renewable energy sources.

- The Severn tidal barrage scheme should be put in hand urgently and should take precedence over nuclear programmes.

- Priority in other new sources should be given to wave power and solar energy and wind power should not be neglected. A target of producing 100 million tonnes of coal equivalent by the year 2000 from renewable sources should be set, and the capital committed to implement it. Most of this can come from the £24 billion it will not be necessary to spend on the nuclear programme. It is of the greatest importance that manufacturing industry be directly involved in the development of these energy sources so that they do not decline into academic laboratory studies, but that commercial application is kept well to the fore. The NEB should be used as a source of capital to stimulate private enterprise.

Three final points

Energy policy should not disregard the world market for power equipment, whether it is to mine coal, extract offshore oil and gas or make use of the sun, wind and waves. It is essential that British industry be geared up to take its share of the unlimited worldwide opportunities that will arise over the next two decades.

In considering policy options for energy there is another aspect of the whole problem to which not enough attention has yet been given, namely: is it sensible in the future to go on building huge power units or would it not be better to think in terms of smaller more dispersed sources of power?

The advantages of this second option would be shorter planning and construction time, greater flexibility for example in the design and construction of combined heat and power systems, less environmental intrusion in some cases, less hostage to fortune in case of failure or breakdown of a particular unit and greater ease of changing policy in the light of new technology. There are exceptions—it would not be possible to build the Severn tidal barrage as a series of small schemes.

Finally, we should not forget the needs of the poorest countries of the world. In many cases they are geographically precisely the countries where the sun, wind, waves, tides and natural hot springs offer the most exciting possibilities for harnessing infinitely renewable energy sources. In some of them, like Zambia, Tanzania, the Congo, the Sudan, the immense distances between towns and villages would make prohibitively expensive and unusable long distance transmission systems, but they would find suitable localised self-contained power units such as aerogenerators, solar power units or wave power units for ports and coastal settlements.

We should not assume that by turning our back on nuclear power we are thereby dismissing the needs of the poorest section of mankind. □

US embargoes nuclear fuel exports to Europe

THE United States is refusing to issue any further licences for the export of nuclear fuels to the member countries of the European Economic Community until these countries agree to renegotiate the current agreement between EURATOM and the US covering the terms of such exports.

Although the embargo covers all nuclear fuels, as well as power station equipment, it is likely to have a particular impact on nuclear research and development in France, Germany, Denmark and Holland which rely almost entirely on the US for supplies of highly enriched uranium.

The American move follows the signing by President Carter on 10 March of the Nuclear Non-proliferation Act, which lists a number of conditions under which the US is prepared to export nuclear fuels. This is aimed at reducing the danger of the proliferation of nuclear weapons. Under the terms of the act, all the US's existing export agreements were to be honoured provided the other party involved indicated within 30 days their readiness to renegotiate existing agreements in line with the new act.

So far, however, EURATOM has not given any indication that it is prepared to renegotiate its existing 20-year treaty. This has been largely because of complaints—particularly from the French—that the US has no right to break the agreement unilaterally, and the feeling that no action should be taken before the conclusions are known of the International Nuclear Fuel Cycle Evaluation (INFCE) which was established last year on President Carter's own initiative, and which is not expected to report until the end of 1979.

In an attempt to minimise any embarrassment caused by the embargo, the US Nuclear Regulatory Commission (NRC) passed all pending licence applications whose paperwork had been completed on 7 April, the last working day before the embargo came into effect. But a number of applications for nuclear fuel were not completed in time. Among them was one from EURATOM's high flux reactor at the Petten Research Centre in Holland for 22.6 kg of highly enriched uranium, submitted to the US last June.

Although the Petten application had been approved by both the Executive Branch and the State Department at the beginning of March, it has been held up within the internal NRC review process, along with two other applications for nuclear fuel from the Netherlands. NRC officials say that

the applications lacked sufficient formal confirmation that the Dutch plants satisfied the criteria on 'physical security' which are contained in the new non-proliferation act.

However other sources in Washington say the NRC is concerned about possible threats to plutonium caused by the recent activities of the Moluccan terrorists in Holland. It has also been implied that any application for nuclear fuel from Italy would have little chance of success in the present political climate.

With the passing of the non-proliferation act, the role of the NRC, which is responsible for seeing that any application for nuclear fuel meets the safeguard and security criteria expressed in the act, has taken on a particular significance in international relations.

In the more overtly political arena, President Carter is now treading a cautious path. He has, for example, maintained a diplomatic silence on the conclusions of the Parker report on reprocessing at Windscale, despite the fact that the report rejects much of the logic of the President's stance.

Even a letter from a number of key congressmen on the non-proliferation issue—including Senator John Glenn and Representatives Clement Zablocki and Jonathan B. Bingham—claiming the proposal to expand reprocessing at Windscale and La Hague in France to be "dangerously premature and obviously inconsistent with the INFCE initiative", was unable to stir the President into making a strong statement. The State Department, too, having been criticised for appearing to be attempting to interfere with the internal politics of other states (for example, in its submission to the UK government on Windscale) is being careful not to tread on too many toes.

In this situation, focus has come to rest on the NRC. And the sensitivity of its role was illustrated by last week's decision by the four commissioners, as the result of a tied 2-2 vote, to reject an application from India for fuel to be used in the Tarapur nuclear power reactor. The two commissioners who voted against the application, Peter Bradford and Victor Gilinsky, said they felt the export licence did not meet three of the six requirements of the non-proliferation act, covering safeguards, the possible use of plutonium to make explosives, and US control over reprocessing. The commissioners voted unanimously that the licence application should be forwarded to President Carter, who can approve it if he finds it in the national security interest of the US to do so—even though such a decision can be subsequently overturned by congress.

Officials in Washington are hoping to avoid a similar confrontation with the member countries of EURATOM. But much depends on the outcome of discussions taking place in Brussels this week on an EEC response to the US demands for renegotiation of the EURATOM agreement.

Dr Guido Brunner, EEC Commissioner for Energy, met French representatives last Monday in an attempt to calm the initial hostility that the US moves have generated, and it is expected that a letter will soon be sent to Washington in which the EURATOM countries, while not directly agreeing to renegotiate as such, will at least indicate that they are prepared to 'talk' about renegotiation.

They hope this will be enough to get the embargo on the export of nuclear fuels lifted, at least temporarily. But the subsequent negotiations promise to be long and acrimonious.

David Dickson

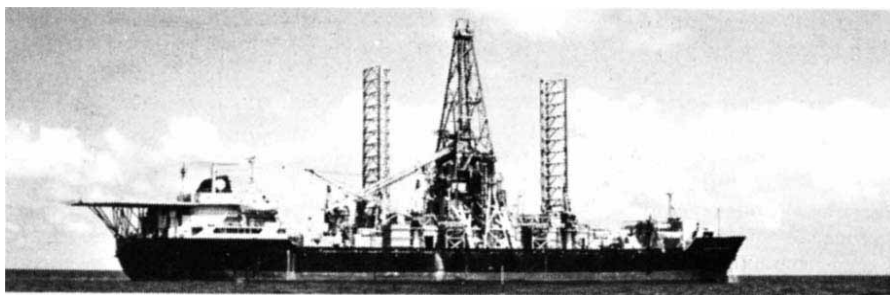
California rejects major nuclear power plant

A LEGISLATIVE committee in California last week rejected legislation that would have permitted the construction of a \$3 billion nuclear power plant because of the problems of the safe disposal of nuclear waste.

Supporters of the Sundesert power plant, presenting a case that even environmentalists accepted was stronger than other applications being made elsewhere, pointed out that it would have been built far from urban concentrations, and was the only pending nuclear power plant in the US that had received both federal and state clearance for site safety including earthquake safety.

However, the state assembly's Resources, Land Use and Energy Committee said it had not been convinced that the supporters had a completely feasible scheme for the safe disposal of nuclear waste, even though the state senate had approved the exemption of Sundesert from the state's waste-disposal laws.

The decision represents a victory for Governor Edmund (Jerry) Brown and his attempts to lead the state away from a nuclear future. Alternatives that Governor Brown is particularly enthusiastic to support include the construction of a coal-fired plant and the repowering of an existing plant. □



Glomar Explorer: bound for seabed surveying?

Congress puts NSF deep sea drilling project in doubt

THE US House of Representatives, citing concern at the balance between 'big science' and 'little science' projects within the programme of the National Science Foundation, has rejected the foundation's proposals for a major start in a deep sea drilling project which is expected to cost between \$450 million and \$550 million over the next ten years.

In its budget proposals for 1979, the NSF wanted to spend \$4.2 million on design and feasibility studies for a major drilling programme to explore the geophysics of the continental margin. The project would include the conversion into a geophysical survey ship of the *Glomar Explorer*, originally built by the Howard Hughes Corporation for the Central Intelligence Agency at a cost of more than \$200 million, but used only once in an attempt to recover a Soviet submarine from the floor of the Pacific.

In its debate last week on authorisation of the NSF's budget for 1979, however, the House of Representatives accepted the recommendation of its Science and Technology Committee, under the chairmanship of Mr Olin Teague, that only \$1 million be authorised for expenditure on the project in 1979. In making this suggestion, the committee said that, although it was not opposed to the drilling project as such, it felt that the case had not been adequately made for including the full expenditure of the \$4.2 million in the 1979 budget. An amendment offered by Congressman Breaux of Louisiana which would have restored the extra \$3.2 million was rejected by the House by 291 votes to 111.

The committee's recommendation was made in the light of its concern about the relative balance of 'big science' and 'little science' in the NSF's programmes. It suggested the NSF should establish a formal procedure for deciding the priority of different 'big science' proposals before undertaking further large expenditure on any new ones.

The committee said the NSF's request for \$4.2 million for starting the deep sea drilling project "contained no indication that the total cost of the programme was estimated at \$450 million over 10 years", and there was no discussion of alternative big science projects which might also need support, such as the "next large telescope". It also suggests that Congress should consider requiring the foundation to request specific authorisation for each new 'big science' project, as is currently done by the National Aeronautics and Space Administration (NASA).

The total NSF authorisation for 1979 recommended by the committee and accepted by the House by 364 votes to 37, is \$934.4 million, almost exactly the figure requested by President Carter. This figure represents an increase of about 8% over this year's budget. Within this overall figure, the authorisation for science education has been increased from \$77.6 million to \$82 million, the committee claiming that it had, as in previous years, been disappointed by the low level of the NSF's request.

To compensate for this, the committee says that, while it applauds the president's decision to strengthen support for basic science in 1979, it "reluctantly" recommends a slight decrease in the amount requested by the NSF for basic research. Included in this category is a \$3.2 million decrease in funds authorised for mathematical and physical sciences and engineering.

Unlike previous years, the NSF's budget proposals have so far gone relatively smoothly through Congress. A resolution to reduce spending on social science research, a stumbling block in the past, was rejected by the House by 174 votes to 229. And in the Senate, Senator Kennedy's health and scientific research subcommittee of the human resources committee last week authorised the NSF's request virtually unchanged.

David Dickson

Lister Institute closes vaccine laboratories

THE Lister Institute of Preventive Medicine announced its plans to close its vaccine and sera laboratories at Elstree last week. The laboratories manufacture most of the diphtheria, tetanus and whooping cough vaccine used in the UK and are the only UK manufacturers of gas-gangrene serum and smallpox vaccine.

The reason for the laboratories closure is purely financial, according to their director, Professor L. H. Collier. The Lister Institute has always funded research from the income earned from its manufacturing activities. Until 1970 this was sufficient to maintain two research units, the one attached to Elstree and a laboratory devoted entirely to research at Chelsea. In addition, the Institute runs the Blood Products Laboratory, also at Elstree, which is wholly financed by the Department of Health and Social Security (DHSS).

Financial difficulty started during the period of high inflation in the early 1970s. In spite of the fact that the vaccine and sera laboratories stepped up their production of vaccines and antitoxins from 12.4 million to 25.4 million doses per year in four years, profits were still not large enough to pay for the research effort. Because the Institute felt that it could only be successful with a successful research programme, in 1975 it decided to close its Chelsea laboratories and concentrate on Elstree.

The plan had been to sell the Chelsea property and to use the proceeds to refurbish facilities and continue funding research at Elstree. After a year on the market, however, the building was not sold. In 1976, the Institute applied to the DHSS for a grant of £½ million to keep its Elstree research facilities running. Last year the grant was refused and the Institute subsequently decided to close the vaccine and sera laboratories.

Production is expected to stop within a few months. But according to Professor Collier it will be the middle of next year before existing stock is despatched and the laboratories can be finally closed. As an economy measure research at Elstree has been reduced since 1974. Since the Lister Institute was established in 1891 it has contributed to many advances in preventive medicine. It produced the first diphtheria antitoxin used in the UK, developed freeze-dried smallpox vaccine, widely used throughout the world, and worked on the transmission of plague, sleeping sickness and trachoma. Recently, however, research has concentrated on rabies and tetanus. Of the 105 employees at the vaccine

and sera laboratories, less than ten are engaged in research work.

The DHSS is confident that any gap in the UK and export demand for vaccines can be supplied by the other two UK manufacturers, Wellcome and a subsidiary of Glaxo. It was decided not to grant £½ million to the Lister Institute because it was reluctant to finance a commercial undertaking, especially when there were other competitive manufacturers, and because it felt that more than £½ million would be needed to fund the Institute's plans.

When the Chelsea property and the vaccine and sera laboratories are finally sold the Institute plans on using the proceeds to further research into immunology. Just how it will do that will not be known until it knows how much money will be available.

Judy Redfearn

Scientists prepare for UNCSTD

At a meeting called by the International Council of Scientific Unions (ICSU) in Paris last January, a steering committee was set up to consider what the non-governmental organisations (NGOs) in science and technology can contribute to the United Nations Conference on Science and Technology for Development (UNCSTD) which is to be held in Vienna next August. The committee has already started organising a symposium which will probably be the most important preliminary to UNCSTD so far as the world scientific community is concerned. It is scheduled for January 1979 and will be held in a developing country—possibly Singapore—where the 70 or so scientists and others being invited will meet for six working days.

The steering committee is chaired by Dr T. F. Malone, director of the Holcomb Institute, Butler University, and convened by Maurice Goldsmith, director of the Science Policy Foundation, London. Little difficulty is anticipated in financing the symposium.

ICSU emphasises that the objective of the steering committee is not to involve scientists directly in the proceeding of UNCSTD, which is now accepted as essentially a political conference, although it is hoped that wherever possible, scientists will be included in national delegations. The aim is also to see what can be done to continue whatever comes out of the conference that is of concern to the scientific community. Put in somewhat pompous officialese, it is "to explore institutional and other innovations which would enlarge and enhance opportunities for scientists and engineers to participate in future years in the contributions of science and tech-

nology to the improvement of the human condition". The stress is on continuing activity, and this is in line with the ideas of such other non-governmental organisations as the Pugwash Conference, the World Federation of Engineering Organisations (WFEO), and the International Social Science Council (ISSC) involved on the outer perimeter of the conference preparations.

Pugwash has already had several meetings in developing countries to examine, amongst other things, the political, economic and even (where the multinational corporations are concerned) psychological problems that may arise for scientists when the conclusions of UNCSTD come to be applied in developing countries. The evolution of guidelines for study at the frontiers of science—especially in agriculture and energy—and the relationship between disarmament and development are other fields being examined by this group in the UNCSTD context.

For ISSC, things are somewhat different. It is felt that the social sciences should have been involved from the first, but in fact it was only at the recent Geneva meeting of the UNCSTD preparatory committee that ISSC appeared as an observer. ISSC feels that its members are only asked to help when the economists and technologists find that their theories are not being accepted or put into practice in the developing countries. Even the UN Development Programme excluded anything like sociology from its projects until recently: now that the fact is being appreciated that development deals with people, as well as economics and industry, the situation is changing. Certainly the experience of the social scientists will be needed if science and technology are to be usefully applied in the developing world.

Peter Collins

Hungary happy with its science

HUNGARIAN science, like that of all Comecon countries, is channelled towards the needs of production. The expansion of R & D needed for the "scientific-technological revolution" in the Hungarian economy made a somewhat slow start, so that in 1964–1969 only 78–82% of the direct non-repayable government grants for R & D were being taken up by the bodies concerned.

A recent report from the Central Committee of the Hungarian Socialist Workers' Party and the Academy of Sciences gives a more promising picture, however. This notes, approvingly, that the "over-all science policy has met the test in practice"



"and if you work hard, young comrade, you may one day get a key to the creators' washroom!"

(which, in Socialist terminology, refers first and foremost to the practice of the factory floor). Some 3.5% of the national income is now being spent on scientific research, while more than 80,000 persons are employed at the various research bases throughout the country.

This expansion, although gratifying to those who compile statistics of national achievements, is apparently causing some problems in practice. A recent broadcast on Budapest radio noted that as Hungarian science is now in a state of transition from "individual sovereignty" to "the necessity of project guidance", the number of scientific personnel is "statistically upsetting".

If the era of classical scientists has now gone by, it asked, is there any justification for the old concessions of flexible working hours, free days and sabbaticals to be granted in the name of "creative freedom"? At present, anyone with a diploma, who works in a research institute, is granted the "status of a scientist"—even if he is only doing routine work, and is "someone who over several years has not produced any evidence; someone who has been searching, but discovered nothing". Why, it is asked, should the "privileges granted to creators" be extended to the "noncreative masses of specialists"?

The basic problem, said the speaker, is organisation. The ideal organiser should not be a stickler for "dictatorial project leadership" but should be someone able to "harmonise scientific objectives with the aspirations of researchers; a person who demands qualitative results, who is not merely of the managerial class, but a scientist who can win the respect of individualistic researchers". Preliminary research has shown that some scientists were disturbed and others stimulated by time-limits imposed on their creative thinking—a "diversity of individuality" which the wise project director would do well to respect.

Vera Rich

Austrian fraud trial adjourned

EARLY this month, the trial of Herbert Schaden, charged with fraudulently obtaining funds for research from a Dutch packaging firm, began in Vienna. But the case may never be settled in court. On the third day, an exhausted Schaden collapsed after 14 months in detention.

The trial continued after Schaden's collapse, until Hertha Celta, accused of having assisted him in the fraud, broke down when the prosecutor showed that some passages from the reports of their work had been copied from someone else's publications without acknowledgement. Celta told the court that she had simply typed what Schaden had given her. However, the court had already decided to halt the case until doctors ruled on whether Schaden was fit enough for further court appearances.

In 1973 Schaden claimed to be on the point of developing a strain of microbe which would digest plastic, but after four years no such strain was developed. From the beginning the case has involved bitter rows between the Austrian Ministry of Science and Research and some science journalists.

The indictment against Schaden cites articles from two Austrian papers and argues that some journalists wrote what Schaden and Celta had told them "apparently without checking its validity". The ministry has repeatedly accused the journalists concerned of being responsible for the loss of research funds worth six million schillings (about £200,000) by the Royal Packaging Industries van Leer B.V. of Amsterdam and 400,000 schillings (£14,000) already paid by an Austrian businessman who was willing to invest 34m schillings in Schaden's inventions. The court adjourned before the 'case of the science journalists'—as the Austrian research minister Dr Hertha Firnberg has dubbed it—could be taken up.

It is clear, however, that van Leer and Dr Roderich Weil, his scientific consultant, got in touch with Schaden because of a Dutch news report based on the Austrian articles. Van Leer said in court that Schaden told him his basic research had been done within the 'International Research Society for Geomicrobiology and Soil Hygiene'. (It was later shown that this is not an international society). Schaden offered him a research programme ready to be scaled up for industrial use. The van Leer group was interested and money started to flow to Vienna.

Van Leer said funds were sent solely on the grounds of what Schaden had told them in Vienna and because the international society and the names of the people involved in it seemed respect-



Schaden on trial (left) and being wheeled from the court room after collapsing

able. Nobody from the van Leer group ever saw the bacteria which Schaden claimed would end plastics refuse problems; nobody ever entered the sterile room in Schaden's laboratory; no specialist in microbiology was sent to investigate Schaden's work before the payments started.

However, van Leer did fly a team of scientists from Israel to Vienna to examine another of Schaden's claims—that he could produce vegetation in deserts by 'bacteriaisation' of the ground. Even though the scientists had a poor opinion of Schaden the money continued in the hope of developing 'plastic-eating' microbes. Funds were only stopped when accounts of how the money had been spent began to arrive late from Vienna and reports of Schaden's work were infrequent and of poor quality.

One piece of evidence which van Leer and some of the journalists saw giving support to Schaden's credibility still remains a puzzle. Dr Heinz Dombrowsky from the University of Freiburg, West Germany, had made a written statement about the high quality of the work done in Schaden's laboratory. Van Leer said in court that Dombrowsky had been invited to Amsterdam when doubts about Schaden started to grow and that he had then been negative about Schaden. But, giving evidence in the Viennese trial, Dombrowsky stood by his earlier opinion of Schaden's ability in microbiology. (Schaden does not have a university degree). Dombrowsky did, however, make the reservation that he knew nothing of Schaden's work on 'plastic-eaters' since his contact with Vienna was over by 1973.

What of the other issues surrounding this case? There is the report written by the science journalist Peter Muller on behalf of the ministry (see *Nature*,

24 November, page 292) which according to the minister was compiled in the hope of preventing any future international ridicule of Austrian science. It was sent to members of parliament and universities and resulted in a heated discussion in parliament. Conservative MPs argued that the 32-page report, financed by the taxpayer, put undue pressure on journalists. They pointed to the assumption in the report that Schaden was guilty in spite of the fact that the court case had not been decided; a point which the minister replied to by saying that everything had already been clearly proved. A Liberal questioned the whole role of the ministry in the affair: if as early as 1973 and certainly as Muller says by 1974 the Ministry thought that Schaden was not to be trusted scientifically, why did it not warn the journalists who continued to write of Schaden's inventions?

These daily skirmishes in parliament partly reflect the stance taken by the opposing parties to the science policy of the ruling social democrats. But there have been some other unusual occurrences so far as science writers are concerned in the Schaden affair.

One of the incidents is particularly unpleasant. A story is spreading that one of the journalists had been paid by Schaden for the articles. A high official of the Ministry for Science and Research repeated the same story during a recent telephone conversation. The facts are that Dr Eleonore Thun-Hohenstein was asked to write a biography of Schaden several months after the first crucial articles had appeared in the *Wochenpresse* in early 1973. She received one payment but backed out of the business because she found Schaden too difficult to work with.

Edith Darnhofer

correspondence

Meaning not sentiment

SIR,—In 'More Comecon cosmonauts' (30 March, page 394) Vera Rich refers to the Russian-Czech joint experiment 'Ekstinktsiya' as an experiment with a "somewhat gloomy name".

I presume that her uneasiness about this name stems from its meaning 'extinction', and from the expression 'to become extinct'. Certainly, this literal meaning does not strike a very cheerful note for the astronauts involved in the experiment. However, in astronomy the word 'extinction' is also used to describe the effect which the earth's atmosphere has on the apparent brightness of a celestial body as it comes nearer the horizon and an increasing portion of its light is absorbed by the earth's atmosphere. In the Ekstinktsiya experiment, this effect is used to study the dimming of setting stars through the extinction of their light in a layer of micrometeoritic dust, thereby revealing the density and particle-size distribution of the latter.

Would it not be advisable that a science writer checks on the wider, technical meaning of a word before attaching to it a label expressing a sentimental value?

MART DE GROOT
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Moralistic fallacies

SIR,—Bernard B. Davis may be right in concluding that scientific enquiry should not be blocked on moral grounds (30 March, page 390) but one or two of his more breath-taking generalisations should not pass unchallenged.

● "In fact, one of the historical grounds for racism was the pre-scientific conception of races as permanent, distinct products of creation. But evolution made us aware of the brotherhood of all races". The most influential pre-scientific account of creation for Western civilisation is in the book of Genesis where all men are descended from Adam and Eve and the whole earth is of one language until men in their pride build a tower of Babel. We did not need Darwin's help to discover the brotherhood of man.

● "... the supernatural basis for a moral consensus was shattered by (Darwin's) elimination of special creation". The Creator's interest in humanity need not be lessened by the possibility that man and the chimpanzee have common ancestors. 'Created in the image of God' is to my mind a better description of man than 'naked ape'. There is certainly something special about the creation of man. Not many of the higher apes read *Nature* or worry about science and ethics.

● "... the rules of behaviour in any society ... are continually evolving". Does evolution here mean progress and if so, what parameters are used to measure it? A few could be suggested: proportion of the society's wealth spent

on armaments, divorce rate, contribution to international terrorism, interest in the starving majority of the world. The word evolving may be fashionable in a behavioural context, but it is empty of meaning.

Dr Davis would probably not agree with me that there is a supernatural basis for morality but he should be very clear about the alternatives. The ground of moral conviction to which he refers is very slippery if it depends on a consensus of human wisdom. Attempts to base ethical systems on the natural sciences are doomed to failure. Evolutionary theory has been used, the good being what survives, but this is merely the worship of success. Monod in *Chance and Necessity*, (Collins, London, 1972) began with the chance origin of life and ended in darkness without any rules. His man-made "ethic of knowledge" requires as much faith as the religious and Marxist systems he dismissed so arrogantly.

Modern science has its roots in the Judeo-Christian view of nature as Hooykaas (*Religion and the Rise of Modern Science*, Scottish Academic Press, Edinburgh, 1972) has shown. When considering science and ethics we do well to remember that Adam was placed in a garden "to till it and to keep it" (*Genesis* 2:15). Perhaps there is a message here for the Friends of the Earth too.

J. N. HAWTHORNE
University of Nottingham, UK

SIR,—If Bernard B. Davis is to convince us that the choice of research should be completely independent of moral considerations he will have to choose his examples more carefully.

The worst that can be said of Bishop Wilberforce is that he was guilty of intellectual dishonesty in trying to ridicule a case that he did not understand. He met his match in Huxley, and Darwin's theories and conclusions were and continued to be fully discussed. He never suggested proscription. The worst that can be said of Urban VIII is that he was provoked by being identified with the naive Simplicio, constantly defeated in argument, into confining Galileo to his home "having had him shown the instruments of torture". It is true that Galileo's works were suppressed in Italy and where the Pope's writ ran but there was no real attempt to prevent *Two New Sciences* being published in Holland. (Really for a Pope he was quite gentle and the offence was mild compared with what he allowed Bernini to do to Rome.) The least evil that might be said of Lysenko is that men died because of his superstitions and his power to enforce them.

Scientists cannot reasonably claim the sole right to judge what risks their fellow citizens may run as a result of their research activities. Would Dr Davis accept that soldiers alone should decide which wars we should have? The division

is the difference between 'risk assessment' and the acceptability of risk and it ought to be.

D. L. SIMMS
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An indifference to facts

SIR,—Careful students of the nuclear energy controversy have long been aware that the popular journalistic media make little pretense of impartiality in their reporting, usually tilting strongly against the development of nuclear energy. Typically their stories display a careless indifference to facts, employing a mumbo-jumbo terminology that is often irrelevant but is nevertheless sure to contain a quota of "scare" words. *Nature*, as befits its distinguished scientific reputation, has hitherto been a refreshing contrast; its news stories have related the facts in detail and with scientific precision.

It is therefore all the more regrettable that the description of the radiation leakage at one of the Belgian plants (2 February, page 398) is filled with errors, of a pejorative tendency, from start to finish. Presumably the incident involved a release of radioactive iodine-131; instead the contamination is described twice as being 'radium-131'. No such isotope exists, but to the lay mind 'radium' is the more horrific term.

Six people were described as "contaminated by 100 millirems" of the substance. Activity of a contaminating quantity of radioactive material is measured in curies. One guesses that what was meant instead was that the people received exposures (external or internal?) amounting to 100 millirems. The report goes on to say that "The maximum tolerated dose was 3000 millirem for 13 weeks before the possibility of thyroid cancer occurred". It is difficult to reconstruct what this could possibly mean. One recognises in the figures an old (and outdated) 1954 regulation which says that for workers in the field a normal weekly maximum exposure of 300 millirems could be exceeded occasionally if no more than ten times this dose were received in a 13-week period ("Permissible Dose from External Sources of Ionising Radiation, Recommendations of the NCRP", NBS Handbook 59, 1954, page 78).

What this has to do with iodine-131 is rather obscure. The concern with iodine-131 is that when ingested in various forms it may concentrate in the thyroid. Recommended radiation protection guides relate to internal exposures at the thyroid or to the permissible levels of activity ingested. The kinds of units used and the levels involved are different from those in the report. In any case there is no question of their setting a threshold beyond which thyroid cancer occurs.

It is to be hoped that the reporting in this story was an aberration, and that *Nature* will in the future resume telling the facts with precision and impartiality.

HERBERT GOLDSTEIN
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news and views

Nuclear antiferromagnetism

from Graham Richards

At the end of March an experiment which truly merits the overworked appellation 'beautiful' was performed at Saclay by Professor Anatole Abragham and his colleagues (G. L. Bacchela, V. Bouffard, M. Goldman, P. Meriel, M. Pineau, Y. Roinel & P. Roubeau); the first direct observation of a nuclear antiferromagnetic structure by neutron diffraction. It was presented at the weekly meeting of the Académie des Sciences in Paris on 14 April and published in the *Comptes rendus* of the same date. For almost 40 years electronic antiferromagnetism has been known: at temperatures below the Néel temperature atomic magnetic moments can form two sublattices with equal and opposite magnetisation. This can be confirmed by bulk magnetic measurements or microscopically by neutron diffraction.

Diamagnetic solids may also have antiferromagnetic structures if the constituent nuclei possess magnetic moments arising from their spins. However, the conditions required to achieve the ordered state of nuclei are considerably more rigorous. Since the interactions between nuclei are only feeble, any orientation effects may easily be disrupted by thermal agitation and the Néel temperatures for nuclear ordering are much lower than those for electronic magnetic moments; within one millionth of a degree of absolute zero.

Although nuclear spins can be

magnetised to nearly saturation by the application of a strong magnetic field of the order of 10 Tesla (100 kgauss) at a temperature of a few millikelvin, the dynamic polarisation method—also developed at Saclay—was used in these, as in previous experiments. In this technique the sample needs to be cooled to only a few hundreds of millikelvin by use of a dilution refrigerator and the nuclear polarisation is achieved by the simultaneous application of a strong steady field and a microwave field acting on the paramagnetic impurities introduced for this purpose. The adiabatic demagnetisation can be carried out either by the reduction of the steady field, or, as was done here by using a radio-frequency field; the so-called demagnetisation in the rotating frame (M. Chapellier *et al.* *C.r. hebdomadaire. Acad. Sci. Paris* **268**, 1530; 1969). Thanks to the slow energy exchange between the nuclear spins and the crystal lattice, nuclear spin temperatures many orders of magnitude below that of the lattice can be maintained for relatively long periods. In this present experiment the antiferromagnetic state persisted for about half an hour.

To prove the existence in the nuclear antiferromagnetic state of two sublattices magnetised in opposite senses one must use neutron diffraction, relying on the fact that the scattering of neutrons depends on the relative orientations of the spins. Purely magnetic interaction between neutron spin and nuclear spin would have a

negligible effect because of the smallness of the nuclear magnetic moment. However it was discovered (Abragham *et al.* *C.r. hebdomadaire. Acad. Sci. Paris* **274**, 423; 1972) that thanks to nuclear interaction, the nucleus behaves as if it possesses a large pseudo-magnetic moment, and effects similar to those found in the electronic antiferromagnetic case are observed.

The spins of the nuclei ^7Li and ^1H in LiH were dynamically polarised in a field of 6.5 teslas produced by a superconducting solenoid followed by adiabatic demagnetisation in the rotating frame. The existence of the nuclear antiferromagnetic structure was then demonstrated by the observation of antiferromagnetic superstructure Bragg reflection from the 110 plane of the crystal. Successive planes have the nuclear spins pointing in opposite directions, so that if we neglect the small differences in nuclear moments of ^7Li and ^1H we can consider the crystal as a simple cubic lattice. The Bragg reflection is absent before nuclear demagnetisation but appeared immediately after demagnetisation and remained for about 30 minutes (corresponding to the nuclear spin-lattice relaxation time) until the long-range antiferromagnetic order faded.

The Saclay group believe that the direct application of neutron diffraction opens a new avenue for the exploration of nuclear antiferromagnetism and more generally of ordered nuclear magnetic states of all types. □

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T and B cell hybridomas

from Elizabeth Simpson

FURTHER progress in the production of 'immortal' lines of functional T or B lymphocytes was reported at a workshop held recently at the National Institutes of Health*. Normal T or B lymphocytes can be 'immortalised' by fusing them with appropriate T or B cell tumours. The isolation of these

monoclonal functional T or B cell 'hybridomas' is allowing an examination of certain aspects of the immune system and the antigens to which its responses are directed, in a way not previously possible.

The initial experiments of Köhler and Milstein (*Nature* **256**, 495; 1975) established that cells from a mouse myeloma could be fused with splenic

B cells from a mouse recently stimulated with antigen, and that a proportion of the resulting hybrids secreted antibodies specific for the stimulating antigen. Subsequently Goldsby *et al.* (*Nature* **267**, 707; 1977) reported that a T cell tumour would

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*Held on 3-5 April 1978, and organised by Drs M. Potter and F. Melchers.

fuse with primed T cells and produce hybrid lines displaying some of the characteristics of each parent.

In general, like fuses with like, and there are ontogenetic and phylogenetic restrictions on the types of cell which will fuse, especially in respect of obtaining stable functional hybridoma lines. Mouse myeloma cells will fuse readily and selectively with both mouse and rat B cells activated by antigens or mitogens. Following hybridisation it is possible to analyse the response to complex antigens, by isolating a range of separate clones, each of which represents a monoclonal response to a single determinant.

This has allowed H. Koprowski and his colleagues in Philadelphia to probe the extremely complex composition of influenza and other viruses with a battery of monoclonal antibodies. The use of antibodies in this way allows the sort of analysis which would have taken an immense amount of time and effort by biochemical methods alone. Histocompatibility antigens have now been used to raise anti-mouse H-2K and H-2I antibodies (G. Hämmerling, Cologne University; P. Jones, Stanford University; U. Hämmerling, Sloan Kettering Institute), anti-rat H-1 antibodies (J. Howard, Institute of Animal Physiology, Babraham; T. McKearn, Chicago University) and anti-human β -2 microglobulin antibodies (M. Trucco, Basel Institute). The titres of anti-H-2 antibodies produced by hybrids growing in culture have been increased up to 100-fold by growing them *in vivo* in mice. Monoclonal antibodies to histocompatibility antigens are extremely useful reagents and it seems only a matter of time before a comprehensive battery of them will be available.

The workshop discussed the problems of making available hybrid cell lines and monoclonal antibodies, and there were several views about how this might be done. The problems arise when the number of requests becomes far too great for any laboratory to satisfy without serious interference with their normal research work. The two options are to license a commercial pharmaceutical company to manufacture and sell the antibodies and cell lines, or set up production facilities under a contract such as those given by the NIH, under which the products are available free to research workers.

Xenogeneic immunisations against cell surface antigens have been exploited by several groups to obtain monoclonal antibodies against differentiation and tumour antigens. Using mice as responders and human melanoma and colo-rectal carcinoma as antigens, Koprowski has produced hybrids secreting monoclonal anti-

bodies which react with a range of human normal and tumour cells in patterns which suggest that some of them may be against tumour-specific antigens. D. Gottlieb (Washington University) has immunised mice with chick embryo brain and heart, and from subsequent hybrids has produced monoclonal antibodies which may define tissue specific and cell type specific antigens, of particular interest in analysing the complex make-up of brain tissue. T. Springer (Harvard University), following immunisation of mice with rat lymphoid cells has obtained hybrids secreting antibodies against a range of cell surface antigens, some not previously recognised by conventional immunisation.

Fusions between 'non-like' cells occur at a very low frequency, and few, if any, of the hybrids which grow retain function as measured by secretion of antibody or by cell surface markers. Thus the mouse myeloma P3X63Ag8 fuses with mouse T cells, but the hybrids neither secrete the myeloma protein nor express Thy 1 antigen (Hämmerling). Fusions have been performed between B cells of widely disparate species, such as mouse-rabbit, mouse-man or mouse-frog (P. Hengartner, Basel Institute). Some mouse-man hybrids secrete human heavy and light chains and from an analysis of 44 such hybrids with

respect to the human chromosomes they retain. R. Levey (Stanford University) is attempting to assign the human Ig genes to particular chromosomes.

T-T cell hybrids have been made in several laboratories, mostly using the AKR T cell lymphoma BW5147 as the tumour parent. Many, but not all express Thy 1, H-2 (K/D and I) and Ly antigens coded by the genome of one or both parents (L. Herzenberg, Stanford University; E. Simpson, Clinical Research Centre, Harrow; J. Miller, Walter & Eliza Hall Institute, Melbourne; N. Ruddle, Yale University; Hämmerling) but the presence of Thy 1 antigen contributed by the normal parent may not be a definite indication that it was a T cell (Herzenberg) and the expression of Ly antigens is not always in accord with those expected from the function displayed by the hybrid (Simpson).

So far nobody has succeeded in rescuing cytotoxic function from T-T hybrids but three groups now have hybrids derived from fusions using as the normal parent T cells stimulated and selected to express suppressor function. These hybrids either show specific antigen binding (Ruddle) or specific suppression in assays performed *in vivo* (Miller) or *in vitro* (Simpson).

□

How Ninetyeast Ridge formed

from Peter J. Smith

THE Indian Ocean has the most complicated tectonic history of any of the world's oceans, a consequence of its origin in the complex breakup and dispersal of Gondwanaland which began during the early Cretaceous. Dominating the topography of the eastern part of this ocean is Ninetyeast Ridge, a 4,500 km long aseismic ridge which lies more or less along longitude 90°E between 32°S and 10°N, is 50–100 km wide and has an average height of about 2 km above the surrounding ocean floor. (For geographic reference, the longitude passing through the southern tip of India is 77°E.) Bathymetric profiles show that the ridge is asymmetric, having along its eastern edge a scarp which rises from a fracture zone.

So what produced Ninetyeast Ridge? At various times it has been regarded as a horst, the result of overriding

plates, a consequence of excess volcanism at the junction of a transform fault and a migrating spreading centre, and the trace of a plume-produced hot spot. Of these, the first two may now be ruled out; gravity and seismic data suggest that the ridge is almost compensated isostatically, a situation inconsistent with a horst or overriding plate. The third was proposed by Sclater and Fisher (*Bull. Geol. Soc. Am.*, **85**, 683; 1974) who also suggested that the fracture zone along the eastern edge of Ninetyeast Ridge is an old transform fault which once marked the eastern boundary of an Indian plate much smaller than the present Indian-Australian plate. The fourth assumes that the ridge formed as the Indian plate drifted northwards over a mantle plume-hot spot fixed in the mantle.

It is this last which Peirce (*Geophys.* **52**, 277; 1978) now supports as a result of an impressive palaeomagnetic study of DSDP cores from on and around

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Ninetyeast Ridge, study which also confirms that the eastern fracture zone is an old plate boundary. One consequence of the plate boundary theory is that Ninetyeast Ridge must always have been attached to the old Indian plate, implying that palaeolatitudes obtained from Ninetyeast Ridge should always agree with the corresponding ones from the Indian subcontinent but should disagree with those from DSDP sites to the east of the ridge for the period before the fusing together of the Indian and Australian plates.

They do on both counts. The new palaeolatitudes from both basalts and sediments indicate that Ninetyeast Ridge moved northward with respect to the south pole at an average rate of $14.9 \pm 4.5 \text{ cm yr}^{-1}$ from 70 Myr ago to about 40 Myr ago when it slowed to $5.2 \pm 0.8 \text{ cm yr}^{-1}$. These rates of absolute motion, resulting in a total movement of about 5,000 km since the late Cretaceous, are in good agreement with the relative rates of closure between Asia and India estimated by Molnar and Tappanier (*Science* **189**, 419; 1975) on the assumption that Eurasia remained almost stationary, and are entirely consistent with the palaeolatitudes from India's Deccan traps. In other words, the rates of motion of Ninetyeast Ridge may be regarded as applying to the old Indian plate as a whole. On the other hand, these rates are inconsistent with the palaeolatitudes determined from a DSDP site 400 km east of Ninetyeast Ridge, confirming that there must once have been a plate boundary between this site and the ridge.

But to return to the origin of Ninetyeast Ridge, both the hot spot and migrating spreading centre hypotheses are consistent with the northward drift of the Indian plate. Where they may be distinguished, however, is in their consequence for the basement palaeolatitudes along the length of the ridge. For a hot spot which was more or less fixed to the mantle, basement palaeolatitudes should be constant, or nearly constant, along the whole ridge. For excess volcanism, on the other hand, basement palaeolatitudes should vary with the varying latitude of the migrating spreading centre. Specifically, for the sites studied by Peirce the ridge basement palaeolatitudes should vary between 55°S and 30°S with a migrating spreading centre, whereas they should be constant at about 50°S if the ridge was formed by the postulated Kerguelen hot spot and at about 40°S if it was formed by the Amsterdam-St Paul hot spot.

Peirce shows that the basement palaeolatitudes throughout almost the complete length of Ninetyeast Ridge are about 50°S , indicating that the

Split gene transcription

from Bob Williamson

THE function of the non-coding inserts found in a range of eukaryotic structural gene sequences is still unclear, but it is now known that at least some of these intervening sequences are transcribed into RNA in the nucleus and then excised at a later processing step. S. Tilghman, D. C. Tiemeier and P. Leder of the National Institutes of Health, and P. Curtis and C. Weissmann of the University of Zurich have shown (*Proc. natn. Acad. Sci. U.S.A.*, **75**, 1309; 1978) that the non-coding insert found in the mouse β -globin gene is present in the 15S nuclear precursor of β -globin mRNA.

Leder and his colleagues had previously demonstrated by hybridisation that cloned genomic β -globin DNA contained a 550-nucleotide long sequence that was not present in cytoplasmic β -globin mRNA. On annealing with β -globin mRNA the non-coding sequence on the genomic DNA does not hybridise and can be seen as a characteristic R loop in the electron microscope. The DNA insert is at the position corresponding to amino acids 104–105 of β -globin. When the same

clone of genomic DNA is annealed to the 15S nuclear precursor of β -globin mRNA, prepared by Curtis and Weissmann by sophisticated affinity chromatography techniques, a continuous R-loop is seen, with no intervening segment. This can only occur if the 15S precursor RNA contains the transcript of the insert.

As the authors comment, an accurate mechanism is required to cleave the transcribed insert from the HnRNA and to rejoin the mRNA sequence accurately. There is little evidence for amino acid sequence errors which might be caused by a failure to rejoin these segments precisely. Some inserts such as those in ovalbumin genes are more than 5,000 nucleotides long, and it is not clear yet whether these are transcribed, nor is it known whether a new class of specific nucleases and ligases must be postulated. Sequence data on the inserts will soon be available and should give clues to the mechanism of processing the primary transcript to functional mRNA.

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source of the Ninetyeast Ridge material must have remained stationary, or almost stationary, for 60–70 Myr. The hot spot hypothesis would therefore seem to win, notwithstanding a strong piece of circumstantial evidence against it. For the close association of Ninetyeast Ridge with the fracture zone along its eastern edge is demanded by the transform fault-migrating spreading centre hypothesis but is only a coincidence in the hot spot theory. Is it likely that the Indian plate would move northwards in such a way as to keep a hot spot trace very close to the bounding transform fault for a distance of several thousand kilometres? It is possible but hardly likely. Yet if Peirce's palaeomagnetic data and his interpretation are correct, as they seem to be, the unlikely would seem to have occurred.

Certainly there seem to be no geophysical or geological data from the vicinity of Ninetyeast Ridge actually inconsistent with ridge formation by hot spot, even though other interpretations cannot be ruled out definitely. One curious feature, however, is the ridge which exists on the adjacent Antarctic plate as a 'mirror image' of Ninetyeast Ridge across the Indian

plate-Antarctic plate boundary for the period 80–40 Myr ago. Again, this is precisely what one would expect from excess volcanism at the junction of the Indian-Antarctic spreading centre and the Ninetyeast transform fault, for it is likely that such excess volcanism would straddle the spreading centre rather than remain on one side.

If, on the other hand, this mirror image ridge is to be explained by a hot spot, it is necessary to conclude that the hot spot remained close to the Indian-Antarctic spreading centre, thereby forming traces on both the Indian and Antarctic plates. And since the hot spot was fixed, the Indian-Antarctic spreading centre must have been fixed too. Yet throughout this period the Indian plate was moving rapidly northwards whilst other evidence suggests that the Antarctic plate was more or less stationary, apparently kept so by forces external to those controlling the Indian-Antarctic plate separation. The inevitable conclusion this leads to is that the accretion of new oceanic lithosphere at the Indian-Antarctic boundary was highly and atypically (at least by today's spreading standards) asymmetrical. □

Forest fires

from Peter D. Moore

It is now well established that fire plays an important part as a periodic disturbing influence on many of the forest types of North America. The species composition of such forests has undergone selection as a result of the regularity of fires during their history so that the vegetation can be regarded as adapted to fire conditions. The same can be said of certain European vegetation types, such as *Calluna* (heather) dominated heathland in the north west of Europe. Regular fires not only influence the composition of vegetation by eliminating sensitive species, they also disturb nutrient cycles within the ecosystem and can lead to considerable nutrient losses. For example, Allen (*J. appl. Ecol.* **1**, 327; 1964) estimated that a single moorland fire could result in the loss of 45 kg ha⁻¹ N, 5 kg ha⁻¹ S (both in smoke) 1 kg ha⁻¹ K (leaching and smoke) and 0.1 kg ha⁻¹ P (leaching). This mobilisation of elements by fire must result in increased nutrient inputs to adjacent ecosystems. In the case of elements lost in smoke, there may result a widespread addition to the nutrient inputs by precipitation and dust among surrounding ecosystems; for leaching losses, the recipient ecosystems will be associated valley mires, streams, ponds and lakes.

The movement of nutrients between ecosystems has been monitored by Wright (*Ecology* **57**, 649; 1976) for small lakes in northwestern Minnesota, following a forest fire in the region. Using a nearby lake from an unburned area as a control, he was able to estimate changes in nutrient input to the test lakes as a result of burning. Runoff increased by 60% due to loss of vegetative transpiration and interception of rainfall. Potassium movement increased by 265% and phosphorus by 93%. Export of Ca, Mg and Na from the burned area were not significantly raised. The fact that the fire occurred in spring, when regrowth of vegetation (and therefore demand for available nutrients) was rapid may be the reason for the generally small influence of this fire upon nutrient budgets. The total phosphorus capital of the lake increased by only 38% which falls within the normal year to year variation. If the fire had occurred in Autumn it might have had greater effects.

Much also depends upon the general morpholometry of the lake basins. A recent study of Green leaf Lake in Algonquin Park, Ontario, by Cwynar (*Can. J. Bot.* **56**, 10; 1978) shows this effect, for the western part of this lake

is surrounded by steep hills and cliffs. A sediment core was taken from the western end, beneath a 90-m cliff, and it was found that the varved sediments showed coincident peaks of charcoal, aluminium and vanadium. The former provides direct evidence of fire (though airborne charcoal could be derived from other basins), whilst aluminium and vanadium probably relate to soil erosion into the lake following removal of the plant cover. Cwynar used samples which span 10 varves (therefore years) in thickness, so the analysis tends to smooth the true curves. A reduction in the thickness of each sample could have provided greater resolution in the quest for evidence of past fires. The study of such sediments gives an indication of the frequency of fires during the history of the basin.

The upper 100 cm of sediments covered a time span of 1,200 years and detailed chemical and pollen analyses were performed on sediments dating between 760 and 1280 AD (that is, pre-European). Within this part of the core there were six distinct peaks in charcoal, the intervals between them were 20, 90, 90, 80 and 100 years respectively. These peaks showed a positive correlation with total pollen influx (presumably supplemented by inwashed soil), aluminium influx and varve thickness. No note was made of the state of preservation of pollen grains, whether corroded or crumpled. Birks (*New Phytol.* **69**, 807; 1970) has used the proportion of crumpled pollen grains as an index of inwash in Scottish lakes, and this could have supplied useful additional evidence in this study.

Conventional pollen analysis of the core showed very steady proportions of the major forest components, pine and birch. There was no correlation between charcoal peaks and pine: birch ratio in the pollen content of the sediments. The conclusion from the study is, therefore, that the fire frequency is about 1 every 80 years in this basin, but that the general spectrum of vegetation remains unchanged by this regular perturbation. The lack of response to fire in the pollen input could be due to a number of factors, such as the 10-year pooled samples, the strong influence of regional pollen rain and the resuspension and resedimentation which occurs in the lakes (see Davis *Science* **162**, 796; 1968). Evidently both the forest vegetation and that of the lake exist in an equilibrium which experiences occasional pulses of biomass destruction and nutrient inflow respectively.

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Pleistocene land-sea correlations

from I. J. Smalley

At least seventeen glacial cold periods and seventeen interglacial periods occurred in Europe during the past 1.7 million years (Kukla *Earth Sci. Rev.* **13**, 307; 1977), eight glacial and eight interglacial periods occurring during the Brunhes magnetic period (about the past 700,000 years). These observations contrast strikingly with the classical Alpine and North European division of the Pleistocene period where only four glacials and three interglacials are recognised. The classical stages can now be correlated with continuous oxygen isotope records from oceanic sediments using loess sections and loess terraces as a link. On this evidence Kukla notes that the terraces representing the four Alpine 'glacial' stages (the famous Gunz, Mindel, Riss and Wurm) fully cover the past 0.8 million years but correspond to both glacial and interglacial climates; the Alpine 'interglacial' stages do not represent episodes of interglacial climate but probably intervals of accelerated crustal movements; the physical evidence on which the North European classical subdivision is based accounts for only about 15% of the time represented. This has led to severe miscorrelations.

Kukla urgently recommends the abandonment of the classical terminology in all interregional correlations and the basing of the chronostratigraphic subdivision of the Pleistocene on the ¹⁸O record of deep-sea sediments. Oceanic sediments reveal the extent of land glaciation because the isotopic variations of oxygen in benthic foraminifera are controlled predominantly by fluctuations in the total amount of land ice and thus give a good record of climatic change which can now be dated with improved accuracy.

Most of the land-based stratigraphic data has come from the examination of sections in the loess at Brno in Czechoslovakia and Krems in Austria (Fink & Kukla *Quaternary Res.* **7**, 363; 1977). Because of their sensitivity in recording regional environmental changes the loess soils of Central Europe have been extensively studied. The Krems loess is older and defines the immediately post-Olduvai events, and the Brno loess, overlapping slightly, neatly defines the Brunhes events. The cycles are separated by what Fink and Kukla call 'marklines'—levels of abrupt environmental change which correlate with the terminations in deep sea sediments.

They are essentially the divisions between deposits of wind-blown loess containing cold-resistant snail assemblages.

The snails have provided powerful evidence of climatic change and the data relating to them were supplied by Vojen Ložek in Prague. The *Pupilla* group assemblage is typical of the Krems loess and characteristic of the glacial loess steppe of Central Europe which had a severe continental and seasonally cold climate. This assemblage is not found anywhere in the world today. *Striata* faunas live today in continental environments characterised chiefly by mild prolonged dry intervals. *Helix* assemblages are those bound by habitat to deciduous forests

and indicate a regional climate similar to that of the Holocene or to one even warmer and wetter. These assemblages prove the interglacial character of strata in which they occur. Ložek is still working on the Krems snails and complete faunal lists will be published in a monograph. The use of snails as climatic markers has proved of critical importance in the investigation of the Central European loess and Ložek's contribution has been essential in establishing the new correlations (see Ložek in *Rozprav. Českoslov. Akad. Ved. Rada. Matemat. Prirod. Ved. Rocn.* **86**, ses 8; 1976). □

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Complex patterns of inheritance in human disease

from E. H. R. Ford

If a disease is transmitted vertically through a number of generations in a family, it has usually been taken as axiomatic that this is due to a Mendelian dominant gene, and variation from the expected 50% proportion of affected children of an affected parent ascribed to variable penetrance. Likewise, if a disease shows a marked tendency to occur in a number of members of a family in one or more generations, it has generally been assumed to have a genetic basis. But recent observations on the vertical transmission of infective agents show that neither of these assumptions are necessarily true. Indeed, the stronger the familial aggregation of a condition, in the absence of a Mendelian pattern of inheritance, the less likely may it be that there is a genetic basis (Harper *J. med. Genet.* **14**, 389; 1977).

Much information on this has come from Gajdusek's well-known studies on Kuru, a subacute, progressive and invariably fatal neurological degeneration found in some areas of New Guinea. This was often transmitted through several generations of a family, but although in childhood both sexes were equally affected, there was marked female preponderance in adult cases. Gajdusek originally suggested that transmission was due to an autosomal dominant gene, fully penetrant in heterozygous females, but rarely penetrant in males unless they were homozygous. However, his studies eventually led to

the discovery that the disease could be transmitted to primates by cerebral inoculation of brain tissue from patients, and that the vertical transmission in families was due to the ritual consumption of the brains of the dead, especially by women and children.

This focussed attention on other fatal encephalopathies. Creutzfeld-Jacob disease, which occurs (rarely) in Western communities is clinically rather similar to Kuru, and transmission through four generations in a family has been described. There is now clear evidence that the condition has been transmitted by corneal grafting and by infected instruments used in brain operations. The condition is more frequent in Libyan Jews living in Israel, where it has been ascribed to this community's fondness for eating sheeps' brains. Another, less uncommon condition is Alzheimer's disease, a form of presenile dementia which has a familial tendency (in 10–12% of cases there was an affected parent). It was once thought to be due to a major gene, but it has now been shown that the disease can be transmitted to primates by cerebral inoculation, indicating that a transmissible agent is responsible. However, the pathology may be heterogeneous, since transmission by cerebral inoculation does not occur in non-familial cases. It is possible that the agent in

Kuru, Creutzfeld-Jacob disease and Alzheimer's disease may be the same—and may even be the same as that responsible for the animal diseases of scrapie in sheep and mink encephalopathy (Gajdusek, *Unconventional Viruses and the Origin and Disappearance of Kuru*, Nobel Foundation, Stockholm; 1977). The proof of viral transmission of these conditions has even led Gajdusek to question the Mendelian transmission of Huntington's chorea. However, the relentlessly regular transmission of this condition over hundreds of years, and inability to demonstrate transmission to primates makes Mendelian dominant inheritance almost certain, though some peculiarities of inheritance suggest that non-Mendelian factors are also at work.

The work of another Nobel prize-winner, B. S. Blumberg, on the Australia antigen, Au (Blumberg, *Australia Antigen and the Biology of Hepatitis B*, Nobel Foundation, Stockholm; 1977) shows how complex the interaction of heredity and environment can be. The antigen was first discovered when it appeared as a new precipitin on the interaction of sera from a haemophilic patient and an Australian aborigine, and was first thought of as a simple polymorphic variant which appeared to show autosomal recessive inheritance. However, Blumberg subsequently showed that antigen negative patients with Down syndrome became Au positive during attacks of hepatitis—and eventually that the Australia antigen is an integral part of the hepatitis virus.

Following the discovery of concentrations of chronic liver disease and primary liver cancer in certain Japanese families, it transpired that affected individuals were Au positive. Transmission of liver disease and Au positive was strongly maternal—16/20 of the offspring of affected women were Au positive, but only 1/8 of the offspring of affected males, strongly suggesting perinatal transmission. Since then it has been found that persistence of the antigen is strongly linked with the occurrence of liver cancer in several areas of the tropics. However, hepatitis B is almost ubiquitous in some of these areas, so it appears that host resistance may be more important than the presence of the infective agent. And indeed it has been found that the pattern of persistence of the antigen after infection is compatible with autosomal recessive inheritance. Thus the wheel has turned full circle, to the extent that overt disease due to the persistence of this infectious agent is almost entirely genetically determined.

It seems that the best way of discriminating between an autosomal dominant gene and a vertically transmitted infectious agent is that with

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the latter, maternal transmission predominates over paternal, and a model has been proposed for testing this (Fine *J. med. Genet.* **14**, 399; 1977). The lesson to be learnt is that the fact that a disease is transmitted in Mendelian fashion does not exclude predominant environmental influences; nor does the presence of a transmissible factor mean that inheritance is not genetically determined. These findings may help in elucidating the mode of inheritance in some other human familial conditions, such as Leber's optic atrophy, where geneticists have for many years been puzzled by apparent inconsistencies with known modes of inheritance. □

Twists and turns of DNA

from Stephen Neidle

It has been realised for some while that the DNA associated with a chromatin repeating unit (the nucleosome), has to be folded to about a seventh of its normal length in order to be accommodated within the known dimensions of this particle. As yet we are some way from having anywhere near an experimental answer to this organisational problem through a high-resolution X-ray crystallographic analysis; it is to be hoped that the quality of the nucleosome crystals reported by the MRC Cambridge group, will in time be sufficiently improved for this goal to be attained (see Finch *et al.* *Nature* **269**, 29; 1977).

In the absence of such data, several hypothetical models have been suggested for the stereochemistry of DNA folding. The initial 'kinked-helix' model, that of Crick and Klug (*Nature* **255**, 530; 1975), involves short stretches (20 base pairs long) of standard B-DNA double helix. The base pairs at each twentieth position are partially unstacked, with a concomitant alteration in the conformation of the sugar-phosphate backbone, so that these straight lengths become bent, at roughly right angles to each other. The small negative twist produced in the double helix by this kinking, enables it to be formed in a toroid suitable for being wound around the histone core of the nucleosome. A modified kink model has been proposed by Sobell *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **73**, 3068; 1976), which has been extrapolated from their crystal-

lographic studies on dinucleoside phosphate-drug intercalation complexes; kinking, this time every 10 base pairs, is accomplished by conformational changes somewhat different from those proposed by Crick and Klug.

The other, contrasting, approach to this problem may be qualitatively visualised by simply taking hold of a molecular model of DNA—it is apparent that the double helix has sufficient inherent flexibility for smooth, continuous bending along its axis to be possible. Such an alternative has been recently described by Levitt (*Proc. natn. Acad. Sci. U.S.A.* **75**, 640; 1978), and independently by Sussman and Trifonov (*Proc. natn. Acad. Sci. U.S.A.* **75**, 103; 1978). In both models, DNA is smoothly bent into a 45–50 Å radius superhelix, which is of the right dimensions to wind around the histone core. Sussman and Trifonov established a (computer-generated) molecular model corresponding to this degree of deformation by the step-wise procedure of first giving a (13 base pair) stretch of classical B-DNA a small negative twist, followed by a bending-type transformation; the less-than-perfect stereochemistry obtained at this stage was then improved by a least-squares method involving fitting standard molecular geometries to the fragments. Their resultant 'smoothly-deformed' DNA does not differ appreciably from the B-DNA except that some of the sugar-phosphate torsion angles systematically vary as one progresses along the double-helical chain. Levitt has employed his previously developed techniques of empirical energy calculations. He has taken a 20 base-pair length of B-DNA, and allowed each of the 820 atoms involved to shift to an energy-minimum position, before deforming it into a supercoiled configuration which like that of Sussman and Trifonov, is both stereochemically and energetically plausible. However, the two suggested superhelical DNA structures are remarkably different from each other, at the detailed molecular level (their superhelical parameters, as one might expect, are broadly comparable). Whereas the Sussman and Trifonov deformed DNA still resembles the classical B form in many important aspects, that of Levitt clearly does not, apart from still being a 10-fold double helix. Particularly striking in the latter are the now markedly non-planar base pairs, with a 31° angle between each base. The sugar-phosphate conformation again shows a periodicity dependent upon the residue's position in the chain, which seems, for some variables, to be sequence dependent.

In the same paper, Levitt has also

provided more details on his energy-minimum form of straight DNA (initially mentioned by Finch *et al.*) which he considers to be more representative of DNA in solution than the familiar B type structure, whose detailed character is suggested to be a consequence of crystal packing forces. In contrast to the 10-fold helix, his calculations suggest a non-integral number of base pairs (about 10½) per turn. These base pairs have a markedly propeller-like character (as in his supercoiled DNA), and thus the individual bases become no longer almost perpendicular to the helix axis.

Direct experimental verification of any of these models is unavailable at present, although a recent ³¹P nuclear magnetic resonance study of DNA in chromatin (Kallenbach *et al.* *Nature* **272**, 134; 1978) suggests that all the backbone units of the nucleic acid are roughly equivalent—this favours a smoothly deformed, as opposed to a kinked model of supercoiling. However, whatever hypothesis is ultimately found to be closest to physical reality, the usefulness of these model-building exercises is undeniable in that they demonstrate that DNA can no longer be considered to be a relatively rigid structure. □

Differentiation dissected

by Peter Newmark

IN the reasonable belief that determination of cell lineage is a prerequisite to the understanding of differentiation attempts are being made to dissect, as finely as possible, the separate steps and decision points in various systems. A selection of these systems was presented at a recent conference on the 'Genetic Control of Cell Differentiation and Malignancy', held early this month at Titisee.*

Of particular interest and complexity is the immune system, in which the stages of differentiation are often defined by the appearance of stage-specific surface markers. These are identified by antibodies, whose production has been revolutionised by the availability of hybridoma cell lines producing monoclonal antibody. Yet higher yields come from the ascites fluid of animals with tumours derived from injected hybridoma cells. C. Milstein (MRC Cambridge) described

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*Held on 6–8 April, 1978. A Dr Karl Thomae GmbH International Titisee Conference.

the production, by those means, of different anti-rat thymocyte membrane antibodies which could distinguish T cell subsets. By similar means Milstein and his colleagues have obtained a rather specific macrophage marker and a strong marker for teratocarcinoma cells that also labels some but not all cells at the morula stage of embryogenesis and 2% of spleen cells.

A much-favoured model system for studying differentiation of the haematopoietic system is the Friend erythroleukaemia cell. This cell can be induced to differentiate into a mature erythrocyte by a wide variety of chemicals, the most effective of which is hexamethylene bisacetamide (P. Marks, Columbia University).

The early stage in which differentiation is still reversible on removal of the inducer can be further subdivided by the selection of variant erythroleukaemoid cell lines. One of the earliest biochemical responses to induction is the appearance of a chromatin-associated protein studied both by H. Eisen (Pasteur Institute) and P. Swetly (Ernst-Boehringer Institut für Arzneimittelforschung, Vienna). Named IP₂₅, this protein resembles histone H1 in being located internucleosomally but is chemically distinct. Eisen suggested that IP₂₅ is responsible for closing down the growth potential of the erythroleukaemoid cells so that differentiation can proceed.

The branch of the haematopoietic system of differentiation that has most occupied L. Sachs (Weizmann Institute) is that leading to the appearance of granulocytes and macrophages. A key to this pathway is a protein known as the macrophage and granulocyte inducer (MGI), which induces cultured normal myeloid precursor cells as well as certain myeloid leukaemia cells each to differentiate along the same pathways to mature macrophages and granulocytes. The steps in those pathways have been characterised by the appearance of markers and it has been possible to isolate mutant cell lines whose differentiation in response to MGI is blocked at various biochemically defined stages.

Whereas continued growth of normal myeloid precursor cells requires the presence of MGI, the myeloid leukaemia cells grow in the absence of MGI. Is there a genetic basis for this clear distinction between growth and differentiation? Karyotype analysis of the MGI-responsive myeloid leukaemia cells and of mutants that had lost the ability to differentiate in response to MGI indicated that the responsiveness (and probably the malignancy) was controlled by a balance between genes on chromosomes 2 and 12.

Karyotypic changes may also be

behind the extraordinary variations in gene expression of a cell hybrid reported by M. Weiss (CNRS Gif-sur-Yvette). The hybrid was between mouse melanoma and rat hepatoma cells. In the initial hybrid line there was reciprocal extinction of differentiation markers of both parent cells—pigment production in the melanoma cells, and albumin production by the hepatoma cells. However, in an experiment that has been going on for four years, the reappearance of both pigment and albumin has been observed. Sometimes their reappearance is simultaneous although from different cells in the same culture. Remarkably the albumin has been characterised as murine despite the fact that it was originally expressed in the rat hepatoma parent, indicating that previously silent mouse genes have somehow been activated. During subcloning of the hybrid cells repeated loss and re-expression of the markers was observed. A karyotypic analysis of the various cells indicated that re-expression of either marker was associated with the loss of a small number of chromosomes from the highly aneuploid hybrid.

For those who tackle the problems of differentiation by way of mammalian embryogenesis, A. McLaren (University College, London) emphasised that in several laboratories the focus is now on the importance of pre-existing maternal gene products (messenger RNA or protein). Others pursue their interests through the injection of embryonic carcinoma stem cells from teratocarcinomas into normal mouse blastocysts. A variant on that experiment reported by B. Mintz (Fox Chase Institute of Cancer Research) provides the first step towards an animal model for the Lesch-Nyhan syndrome (*Proc. natn. Acad. Sci. U.S.A.* **74**, 5564; 1977). In another variant K. Illmensee (University of Geneva) described the results of injecting a hybrid cell derived from fusion of a mouse teratocarcinoma cell with a human fibrosarcoma cell. The hybrid retained human chromosome 17 which carries the gene for galactokinase and a search was carried out in the chimaeric mice obtained for the expression of human galactokinase. Weak activity in two tissues of one mouse provided suggestive, but not unequivocal, evidence that human genes can be expressed in the mouse. Evidence for the expression of other human chromosome 17 products, particularly collagen, is being sought.

Finally the tumour virologists had their say. E. Wecker (Wurzburg) put forward the possibility that endogenous retroviruses have a role in the activation of lymphocytes by antigens and mitogens. R. Weiss (Imperial Cancer Research Fund, London) emphasised

the multifarious effects of tumour viruses on the development of cells including the ability of Rous sarcoma virus to convert pigmented retinal cells into lens cells, a process that must involve de-differentiation before the trans-differentiation. Another example, from the work of N. Teich and T. Dexter, is the way in which different viruses 'push' pluripotent bone marrow cultures in different directions; Friend leukaemia virus induces erythrocyte and granulocyte formation whereas Abelson mouse leukaemia virus (AMLV) infection gives rise to lymphatic leukaemia cells (null cells) *in vitro*.

A similar system, in which bone marrow cells are converted into clones of transformed lymphoid cells (immature B cells or null cells) by AMLV and a helper virus has been exploited in the laboratory of D. Baltimore (Massachusetts Institute of Technology). He reported that transformation was accompanied by the appearance of a protein (p120) that consists of only a small portion of the *gag* gene product of AMLV, together with a large fragment of unidentified, but presumably cellular, origin. As with other acutely transforming retroviruses AMLV appears to be a defective transducing virus which has picked up a large insert. The possibility that p120 plays a part in the process of transformation is now being explored.

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A hundred years ago

A LITTLE village in the neighbourhood of Druguignan, France, has lately been the scene of a remarkable subsidence which has attracted the curious from all directions. An elliptical tract of ground, containing over 10,000 square feet, sank gradually one day, accompanied by loud noises, until it left an orifice of over 100 feet in depth, with water at the bottom.

From *Nature* **17**, 25 April, 513; 1878.

BOOK REVIEW SUPPLEMENT

MAN has always been fascinated by his own seven ages, and has used his own life history as an analogy for nearly everything else that has a history, even if it does not have a life; if the macrocosm is mirrored in the microcosm, then surely the macrocosm also should progress from conception to death. Successive creations, kingdoms and empires, even species, genera and higher taxonomic groups, should come to birth, flourish, become senile, and die, like Man himself. With the coming of evolution, these ideas were by no means obliterated. Even though it was realised that the evolutionary analogy was imperfect, that a particular group instead of becoming extinct could give rise to others indefinitely, at least in principle, nevertheless the analogy of the developing individual with the evolving group was probably the most powerful single persuasive to the acceptance of evolution ever produced. Who could boggle over the little change from a monkey-like ancestor to Man, when he himself had begun life as a single cell, effectively a protozoan?

Moreover, the intensive anatomical investigation of early developmental stages produced some outstanding triumphs for the evolutionist: Kowalewsky's discovery of the chordate character of the ascidian tadpole, the elucidation of the comparative anatomy of the middle ear bones, the gill arches or the thyroid, or the indubitably crustacean larvae of barnacles and their degenerate parasitic relatives, for example. Such things are naturally so taken for granted now that it is hard to understand the excitement they caused, the sudden clarity they brought into the true relations of enigmatic organs or groups. When the key to the difficult characters of higher forms can be found so often by comparing early stages with each other, or with the early or later stages of lower forms, who can doubt that there is an intimate relationship between ontogeny and phylogeny? But what exactly is that relationship? The question has never been answered.

Steve Jay Gould has given us a superb analysis of the use of ontogenetic analogy, the controversies over ontogeny and phylogeny, and the classification of the different processes observable in comparing different ontogenies. His massive book (in each chapter of which there is as much material as in whole books by other writers) is both a historical exposition

of the whole subject of ontogeny and phylogeny, and (after a logical analysis of the concepts involved) a fascinating attempt at a functional interpretation of those phylogenetic alterations that involve changes of timing developmental processes in related organisms. This is the major preoccupation of the book, with those evolutionary events that do produce apparent parallels between ontogeny and phylogeny. He shows carefully that although in a particular life history, growth and differentiation may proceed *pari passu* and maturation in-

liable to occur under *r* selection and neoteny under *K* selection.

He is undoubtedly right in seeking to explain such processes as paedomorphosis, or heterochrony generally, in terms of immediate selection pressures. As he says himself (p285), the classical arguments were entirely retrospective in design; they "look upon a case of neoteny after its descendants have evolved and attribute meaning in terms of the aggregate success. But what of the actual species that experienced neoteny?" What, indeed! A population does not start becoming neotenuous now because it sees that in ten million years' time it will give rise to a whole new phylum.

Given that sex is necessary and involves beginning new individuals from a single cell, it follows that the adult stage of a species, however complex, must be developed from that cell. This takes time (presumably for good thermodynamic reasons, though rates vary so much, one has a feeling that they are selected for, not imposed compulsorily) and consequently there is a period in any life history when the organism is merely getting on with developing into something else. The question is, what is the simplest set of operations, involving the most reliable coordinating signals, for getting from a fertilised egg to a man, for example? Even if a man did not have to bend his back, using carefully articulated vertebrae, would it still be the best means of making that part of him to produce a lot of embryonic tissue, break it up into regular masses, get a consequent metameric series of nerve-roots growing out into or between them to ensure complete innervation, and then complicate them into plexuses for specialised purposes? If so, we might well follow our present course of development quite irrespective of what our ancestors were.

In many life histories, the earlier stages are not simply devoted to developing; they may be dispersal or feeding stages with temporary characters adaptive for those ends. It seems to be the case that those characters obviously adaptive are taken as such, and those that are not obviously adaptive are taken as ancestral, or recapitulatory, or at least as anticipatory of later characters. (This is a prime example of what I have categorised before as explaining—in the case of the adaptive characters—and explaining away, since in the latter

Ontogenetic analogy

A. J. Cain

Ontogeny and Phylogeny. By Stephen Jay Gould. Pp. 501. (Harvard University Press: Cambridge, Massachusetts and London, 1977.) \$18.50; £12.95.

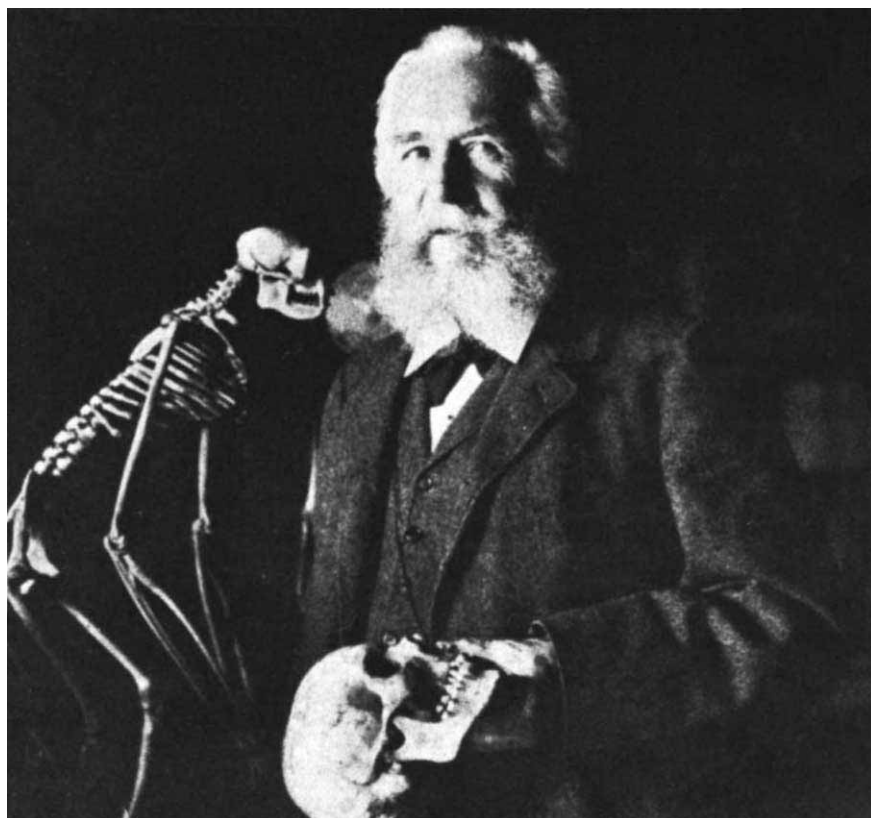
tervene at a particular stage, nevertheless all three processes are different and can be dissociated in evolution, or experimentally. By bringing in a biological clock—actual rate of development in time units where possible, or at least rates relative to major occurrences such as metamorphosis in frog tadpoles or pupation in insects—he distinguishes between different processes previously confounded, as they may give the same relative results.

In particular, he distinguishes clearly between progenesis, with precociously early maturation before growth or differentiation have reached their normal levels, and producing a truncated life-history, and neoteny, in which the life-history takes the normal time (for that group) but somatic development is retarded, as in the full-sized or even giant adult tadpoles of various Anura. Both produce results that can be classed under paedomorphosis, the retention of youthful ancestral features by adult descendants; but Gould suggests convincingly that their significance in evolution is wholly different, that they are produced for totally different life history strategies, and that in fact progenesis is specially

case one has only ignorance not positive knowledge, to work on, as Gould recognises). Such *ad hoc* adaptive features as the placenta were of course recognised as such right from the beginning and discarded in the search for phylogenetic information.

Gould suggests, agreeing with Ledyard Stebbins, that novelties in the developmental history cannot begin early, or they would disrupt it (p268); but if they are found to be advantageous when they appear later on, they will be shifted backwards in developmental time to give greater and greater advantage. This can hardly be the mode of origin of the placenta or of most other larval adaptations, but Gould is concentrating on those features in which heterochrony can be seen in evolution. In particular the very different pressures produced by *r* selection and *K* selection will have this effect. But what is primarily selected for in these cases will be times of maturation and rates of development, not morphological characters. Here, Gould over-emphasises his own point. These may be the primary features, but it does not follow that others are not selected for consequently and efficiently. He says of progenesis: "juvenile morphology is often an incidental (though not inadapative) by-product" (p320); and in relation to juvenilised male mites:

Ernst Haeckel, who formulated the principle that ontogeny repeats phylogeny



Courtesy National Library of Medicine, Bethesda, Maryland

"The partial retention of larval features is a developmental consequence of selection for a life-history strategy involving precocious maturation. These features are not inadapative; they are probably not even "neutral"; but I would be surprised if selective pressures for their evolution played any important role in the evolution of dwarf males" (p332); and again (p338); "morphology is simply not the primary ingredient of many progenetic adaptations. The re-direction of selection towards the timing of maturation might well release the rigid selection usually imposed on morphology. Morphology would then no longer be fine tuned to a changing environment".

Now, if there is selection for efficiency in proceeding from the egg to the adult, and still more if there is for larval adaptation, juvenile characters cannot be viewed as simply the consequences of a re-timing of maturation. The primary change in the selective regime may be for re-timing and may involve huge selection pressures; but consequent ones on juvenile features as such may still involve selection of the order of several per cent. This overemphasis blocks the way to an investigation of what are the selective pressures on juvenile features as such, an investigation as necessary in embryology as one into real values of selection coefficients in wild populations has been for population genetics. The ambiguity in the quotations just given lies in the use of the word "important", and is not characteristic of Gould's lucid style.

The book is excellently illustrated with a variety of examples, each carefully analysed, often with surprising results. Gould explains why progenesis in insects is often not genetically determined, whereas neoteny in salamanders is; he presents a new view of evolution in that classic oyster *Gryphaea*, a model analysis of variation in the snail *Cerion*, some fascinating questions as to what solitary and migratory locusts are really doing developmentally and ecologically, and an excellent analysis of paedomorphosis in Man. Quite outstanding, though, is his treatment of the history of the subject, which is real biological history, not the history of ideas ripped out of their practical context. We are shown exactly why Weismann, contemplating sphingid moth caterpillars, rejected natural selection as the means of producing recapitulation—as a result of biological work, not of historical, ethical or economical borrowings. (But was he right?). It is refreshing also to find an author who has a good word (and the right one) for Ernst Haeckel, who does not treat Charles Bonnet as a stupid monomaniac, who brings out clearly the relationship between acquired characters and recapitulation in the work of the American neo-Lamarckians. Yet the book is not a hybrid of history and science, but a coherent whole, as Gould modestly claims (p5). And remarkably cheap at the price. □

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A World of Naturalists

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JOHN MURRAY

Tortuous story of DNA

Robert Olby

A Century of DNA: A History of the Discovery of the Structure and Function of the Genetic Substance. By Franklin H. Portugal and Jack S. Cohen. Pp. 384. (MIT Press: Cambridge, Massachusetts and London, 1977.) \$17.50, £12.25.

THE title of this book refers to the century spanning the isolation of a curious substance from the nuclei of pus cells in 1869 and the establishment of the structure of DNA and most of the details of the genetic code by 1969. In clear prose, supported by excellent explanatory figures, the authors proceed, in roughly chronological sequence ranging over a wide variety of disciplines, to expound the tortuous and complex story of the development of our understanding of the structure and functions of DNA. They do not claim to have written a "totally detailed history or an academic one", but rather have set themselves the task of explaining to the general reader with a background in advanced-school chemistry and biology why it was that more than a century of research was required "before scientists properly understood what DNA was and what it did within the cell."

The authors are to be congratulated for the many evidences this book shows of care in attention to detail, and for the patience and determination that was undoubtedly required to bring the work to such a successful conclusion. The publisher, too, deserves high praise for the standard of reproduction, clarity of photographs, and for the layout and general appearance of the book.

The book opens with an account of the work of the Swiss physiologist, Fritz Miescher, who isolated a grey powder from the nuclei of pus cells (obtained from discarded surgical bandages). Perceiving its very distinctive properties he concluded it represented a new class of compounds and he called it nuclein. Like the proteins, he saw the nucleins as an important centre of metabolic activity, and he speculated that they acted as a storehouse of phosphorus. His elementary analysis of nuclein was carried out on the product from pepsin digestion of the cells. From its nitrogen/phosphorus ratio we can be confident that this was nucleohistone. When he dissolved his product from acid extraction of the cells, on the other hand, he must have obtained a solution of the sodium salt of DNA. His teacher, Hoppe-Seyler, feared that the pepsin digestion of pus cells had yielded peptones which had combined with phosphatides to give a product with a higher

N/P ratio than was found in the only well known phosphorus-containing organic compound, lecithin. There had only recently been a dispute in his laboratory over the alleged phosphatide from brain tissue called protagon. With two of his research students he set out to verify and extend Miescher's results. This led to delay in publication for Miescher until 1871. A year later Miescher reported to the Natural Science Society in Basel on his isolation of a purer form of nuclein from salmon milt. He separated a basic component from the nuclein to which he gave the name protamine. He realised that in the cell they were held together in a salt-like union.

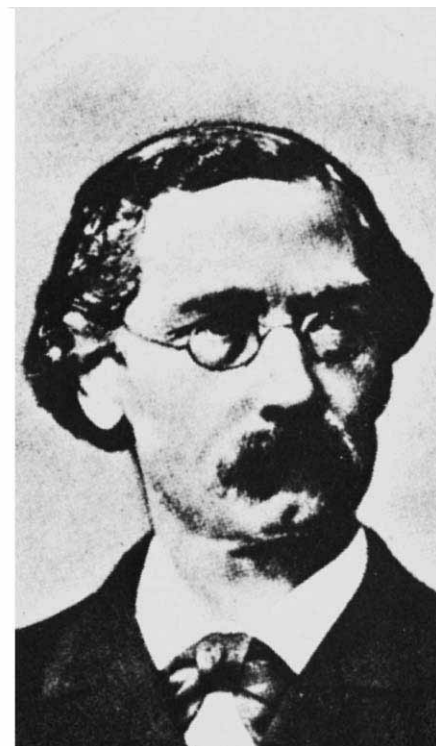


Miescher (left) and Hoppe-Seyler

Both Miescher and Hoppe-Seyler believed that there was some metabolic path connecting the nucleins with the proteins. Lengthy studies of the fasting stage in the male salmon by Miescher seemed to confirm this hope. The build-up of large quantities of nuclein in the gonads came after the progressive "liquidation" of the animal's trunk muscles. Confusion was increased by Nicholas Lubavin who not only claimed to have found nuclein in milk, but also that he had split it into phosphorus and protein. The presence of xanthine bases was observed by Miescher in his studies of salmon spermatozoa in 1872. Had he recognised these bases as constituents of nucleic acid, he would have attained a clear means of distinguishing the nucleins from proteins, but he believed these bases came from protamine.

Thus it came about that Richard Altmann and Albrecht Kossel were able to introduce a new phase of nucleic acid research at the end of the nineteenth

century. Altmann introduced an improved extractive procedure, and showed that nuclein could be separated into a phosphorus-containing component, to which he gave the name "nucleic acid", and a protein component. Kossel and his colleagues identified the distinctive breakdown products of nucleic acid—xanthine, hypoxanthine, adenine, guanine, thymine and cytosine—between 1879 and 1900. The authors mention Kossel's difficulty in deciding whether the first two of these were primary or secondary products of nucleic acid breakdown and they are careful to point out that Kossel was envisaging a variety of nucleic acids, distinguished by the bases they contained. Thus calf thymus nucleic acid was



Courtesy National Library of Medicine, Bethesda, Maryland

thought to contain only adenine, because adenine was the only base extracted from this source. The possession of these bases, however, they recognised as the feature marking off nucleic acids from the 'paranucleins' of egg yolk.

Meanwhile, structural studies of the bases were carried out, on the one hand using the procedure of degradation and identification of fragments, and on the other hand using synthesis. Emile Fischer synthesised the purines in 1897, and by 1903 the two pyrimidines, uracil and cytosine, were synthesised and their relationships discovered. In the first three decades of this century progress in the understanding of the manner of attachment of the constituents of DNA advanced slowly, because of the considerable difficulties of the task, and it was not until 1929 that the identity of the sugar in DNA was established. The merit for establishing the basic structure of DNA's building blocks was chiefly P. A. Levene's. Cohen and Portugal hold that Levene has

been inadequately recognised. His omission from the Nobel awards they consider to be "glaring".

Levene introduced the terms nucleoside and nucleotide in 1909, when he and Jacobs established the sequence phosphate-pentose-purine in inosinic acid. In 1912 they set out a chain structure for which they finally suggested the correct 3',5' phosphate-sugar links in 1935. Levene and other authorities believed that all four nucleotides had a separate existence in the cell—adenylic, guanylic, thymic and cytidylic acids, as well as the combination of all four in DNA. The picture of DNA as composed of these four in a fixed order was the natural result. This tetranucleotide structure implied that DNA was a small molecule with no possibilities for isomerism.

Evidence of depolymerisation of native DNA by enzyme action was found by Feulgen and Levene in the late 1930s, along with the first evidence of the existence of polymeric DNA. Although these findings did not undermine confidence in the tetranucleotide hypothesis which was able to continue as a polymeric tetranucleotide, other studies brought its demise by 1950. These were Avery's isolation of the transforming principle, Gulland's study of DNA ionisation constants and Chargaff's DNA base analyses. The RNA tetranucleotide hypothesis suffered a like fate at the hands of Seymour Cohen and W. M. Stanley. The subsequent phase of structural studies leading to the Watson-Crick model is too well known to need repeating here. Where the authors have made a valuable contribution is in their account of the history of researches on phage. This is a very thoroughly researched and informative part of the book. The feeling of uncertainty as to the way the work would go and the changes and shades of opinion over the biological functions of the DNA and protein in phage, which have already been referred to in earlier accounts, are brought out strikingly by Cohen and Portugal.

The final part of the book is devoted to protein synthesis, the genetic code and what the authors call "the second century of studies on DNA". Interest in the mechanism of protein synthesis evolved slowly. In 1935 the continued turnover of cell proteins both during and after completion of growth was demonstrated by Borsook and Keighley, and confirmed in 1939 by Schönheimer using radioactive isotopes. Next, Fritz Lipmann introduced the energy-rich compound ATP for the linking together of amino acids. Alexander Dounce's remarkable account in 1952 of a scheme for protein synthesis based on a triplet RNA code was followed by Dalglish's suggestion that the growing polypeptide chain peels off behind the zone of formation on the nucleic acid template. The ensuing very active phase of research on protein syn-

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thesis involving the discovery of transfer and messenger RNA, and the main features of the code is covered clearly and competently, and includes extracts from interviews with many of the participants.

The last chapter provides an excellent summary of recent developments in molecular biology: the discovery of two types of DNA, repetitive and non-repetitive, in 1967; of DNA restriction enzymes and satellite DNA in 1968; of three DNA polymerases in 1962, 1969 and 1971; and of reverse transcriptase in 1970. This survey leads up to a grand finale, quoting from Francis Crick: "Between now and the year 2000 biological research will take place on a massive scale . . . In short, the whole field is likely to be even more fascinating in the year 2000 than it is today".

As a contribution to didactic literature on molecular biology this book must earn very high marks. To the scientist wishing to learn about the evolution of our understanding of DNA it is strongly recommended. To the historian it presents some problems. These arise chiefly from the attempt to conceive the account within a period with such a long time span. As a result, the authors have been tempted to look for parallels between concepts introduced within widely different contexts and times. In a subject as fast-moving as twentieth-century biology and biochemistry, looking for links can seriously warp interpretation. In charting a new area it is helpful to set it in the context of several distinct periods. This exposes discontinuities in the development of the science and exhibits phases of major change. One wonders whether, for instance, there is not a marked contrast between studies of protein synthesis before and after the early 1950s. One would like to know much more about such studies in the early period, viewing them in their own right and not as steps leading towards the correct solution.

In their chapter on early theories of heredity the authors refer to nineteenth-century cytologists who discussed the molecular constitution of the hereditary material, and they conclude that these cytologists' interpretations "inaugurated a truly molecular biology". Although it is true that Ernst Haeckel believed the complexity of the protein molecule could account for vital phenomena, his concept of molecules with a capacity for memory, was not very clear, and all the cytologists mentioned by Cohen and Portugal were strongly opposed to Haeckel on this point. For them the chemical molecule was too small and simple to account for the properties of the germinal material. This had to have an organised structure above the level of chemistry; hence their introduction of morphological structures—the biophores, pangenes and micelles. One of Naegeli's micelles in the starch grain would thus have been made up 9,000 chemical molecules. Even the word molecule was not always used to mean the chemical molecule: witness Naegeli's account of the starch grain in 1858. It is arguable that a better historical approach is to set out the contrasts between the ideas of these nineteenth-century cytologists' and those of contemporary molecular biology, rather than to draw tenuous parallels. The macromolecular concept of the 1920s and early 1930s then emerges as a major conceptual development which serves to mark off nineteenth-century 'biophysical/morphological' explanations from the truly molecular models put forward by Przibram (1927), Koltzoff (1928), Goldschmidt (1932) and Muller (1936).

Again, it is of doubtful value to point up similarities between Hugo de Vries' migration of pangenes from the nucleus into the cytoplasm and messenger RNA. About the only feature that they have in common is their migration. De Vries' pangenes need to be considered in the context of other attempts to account for the way in which the nucleus controls events in the cytoplasm. Was it by the transmission of a dynamic vibration, by chemical diffusion, or by ferments? It is true that the basic nucleus-cytoplasm relationship accepted today was perceived by de Vries, but he was in good company with Haberlandt, Nussbaum, Hansen, Korschelt and Strasburger. He, no more than they, envisaged a macromolecular messenger like RNA.

The greatest strength of this book lies in its authors' understanding of the science, which has enabled them to pick their way through the tangle of obscurities and confusions in the complex story of nucleic acid researches. Their success in so doing constitutes a considerable contribution to the history of the subject. □

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Scientist, versifier and prophet

Sydney Smith

Doctor of Revolution: The Life and Times of Erasmus Darwin. By Desmond King-Hele. Pp. 361. (Faber and Faber: London, 1977.) £12.50.

In this magisterial work, Desmond King-Hele does full justice for the first time to the extraordinarily diverse and original gifts of this truly remarkable Englishman. Erasmus is peculiar amongst the bearers of the famous name of Darwin in having the misfortune that his enduring claim to recognition as expositor of the view that all living things had a common origin from a living filament which, diversified through descent with modification, did not need the continuous intervention of a creative designer; seemed invalidated by his grandson Charles who forced the acceptance of a closely similar view by his publication *The Origin of Species* in 1859. The fame of the grandson revived the sagging reputation of Erasmus as scientist versifier prophet, but advocacy from Samuel Butler in the late 1870s was so polemical that it did not command unquestioning support.

Erasmus was a successful doctor with a practice which covered all classes of Midland society. He steadfastly refused to serve the Crown and to move to London. His medical wisdom was published in *Zoonomia* (1794, 1796) which achieved a third edition in 1800 and was translated into French, German and Italian. His other prose scientific work, also published in 1800 and in German in 1801, was *Phytologia; or The Philosophy of Agriculture and Gardening*. Erasmus's fame was established by his works in verse. Starting with *The Loves of Plants* (1789), and followed by *The Economy of Vegetation* (1791), issued together under the title *The Botanic Garden*, it attracted the attention of Wordsworth, Coleridge, Shelley and Keats as well as a witty parody from the *Anti-Jacobin*. His last verse didactic work *The Origin of Society* was published posthumously (1803) under the title *The Temple of Nature*. Erasmus's fame persisted until 1825 when this last work appeared in a third edition. German and Russian translations were made in 1808 and 1911, respectively.

Erasmus's didactic verse presentation of the sexual system of Linnaeus for the classification of flowering plants achieved notoriety from his evident delight in suggestive nuance—in which he followed his model Linnaeus—in his personification of the Stamens and Pistil. Solid instruction in prose was given in lengthy footnotes and appendices. Early editions beautifully printed in quarto, with aquatint and line engravings by Fuseli and Blake among others, demonstrate the unity of Culture in the days before specialisation, jealous diversification and suspicion supervened. King-Hele holds that for Erasmus Darwin "poetry was little more than prettyfied prose, facts robed in finery"; but a finery economical in cut and effectively oriented towards a didactic conclusion. This can be easily illustrated by quotation from C. M. Frere's parody *The Loves of the Triangles* from the *Anti-Jacobin* issues of 16 and 23 April, 1798. Lines 6–10 addressed to the "Sons of War and Trade" read:

"No DEFINITIONS touch your senseless mind;
To you no POSTULATES prefer their claim;
No ardent AXIOMS your dull souls inflame;
For you no TANGENTS touch, no ANGLES meet,
No CIRCLES join in osculation sweet!"

After speaking of the Nymph of the Wheel, TROCHUS, who from her chimney corner longs for the Smoke-Jack of whose attributes Frere urges the reader to (lines 51–58):

Mark how his various parts together tend
Point to one purpose, —in one object end:
The spiral grooves in smooth meanders flow,
Drags the long chain, the polish'd axles glow,
While slowly circumvolves the piece of beef below:
The conscious fire with bickering radiance burns,
Eyes the rich joint, and roasts it as it turns."

"The conscious fire" has a footnote from lines 213–214 of "The Economy of Vegetation". Here, the Sylphs and Genii of fire

"... mark with shining letters
KUNKEL's name
In the slow phosphor's self-consuming flame."

Erasmus's verse lends itself to such establishment parody and his views are pilloried with Godwin's in the letter of introduction allegedly from a Mr Higgins announcing the object of the parody "to rescue and recover the interesting nakedness of human nature, by ridding her of the cumbrous establishments which the folly, and pride, and self-interest of the worst part of

our species have heaped upon her . . . We contend . . . that Institutions civil and religious, that Social Order (as it is called in your cant), and regular Government, and Law, . . . are but so many cramps and fetters on the free agency of man's *natural intellect*, and moral sensibility; so many badges of his degradation from the primal purity and excellence of his nature."

Desmond King-Hele, mathematician and physicist, engaged in research by satellite, poet and versifier, the author of a critical study of Shelley now in a second edition, shares a remarkably congruent range of interest with his subject and is thus uniquely qualified to interpret Erasmus Darwin for us today. His enthusiasm for Darwin already in 1963 led to a work of 174 pages; in 1968 this was supplemented by liberal extracts of the texts called *The Essential Writings* . . . The book under review is enlarged to 323 text pages cleanly and economically printed, annotated by chapter and by page in an appendix. A fully researched listing of Erasmus Darwin's publications, a listing of the nature and location of manuscript material much of it only recently accessible to scholars, and (on pages 326-327) a most useful family tree of Darwins extending over five

generations—two before and two after Erasmus (unfortunately marred by slips in dating. Marianne Darwin was married in 1824, and Caroline in 1837) are included. There are of course overlaps in text and materials with the earlier publications, but with so much so new, so relevant and informative this is entirely excusable. The new discoveries are incorporated with tact and skill into a chronological account in which appear Erasmus's interests and the state of his numerous flock of children, both legitimate (he was married twice) and (after the death of his first wife) illegitimate (though fully acknowledged). The education of these two illegitimate daughters to fit them as governesses led to the publication in 1797 of his *A Plan for Female Education in Boarding Schools*.

The title *Doctor of Revolution* evokes the query: In what sense is Revolution to be taken? Erasmus as parodied by the *Anti-Jacobin* in 1798 was lumped with Godwin, Shelley's father-in-law, and he expressed sympathy for both French and American revolutionaries; though, sickened by bloodshed, his French enthusiasm waned. He never could be regarded as an active participant. There can be no doubt of his importance for the Industrial Revolution as a founder member of the Lunar Society of Birmingham. He was friend and collaborator of Josiah Wedgwood, Matthew Bolton and James Watt, a lifelong friend of the chemist James Keir, correspondent with Rousseau and Franklin, coach and carriage improver with Richard Lovell Edgeworth. But their remarkable ingenuity hardly bore fruit for the railroads ousted road transport too quickly. Likewise, Darwin's horizontal windmill, which ground pigments for Wedgwood's factory for 13 years before being displaced by Watt's steam engine, exists only as a sketch in his *Commonplace Book* now at Down House. It is a great gain to have a survey of the full range of Erasmus's sketches reproduced here. As a proponent of Evolution rather than Revolution, Erasmus was too much of a physician to advocate violent change. The semantic and historical problem of what the word Evolution means is too large a topic for now.

In sum, this persuasive account of the elder Darwin is a landmark unlikely to be superseded. It is a pity that contemporary costs of production may price it beyond the means of the private buyer. □

Sydney Smith is Lecturer in Zoology at the University of Cambridge, UK.

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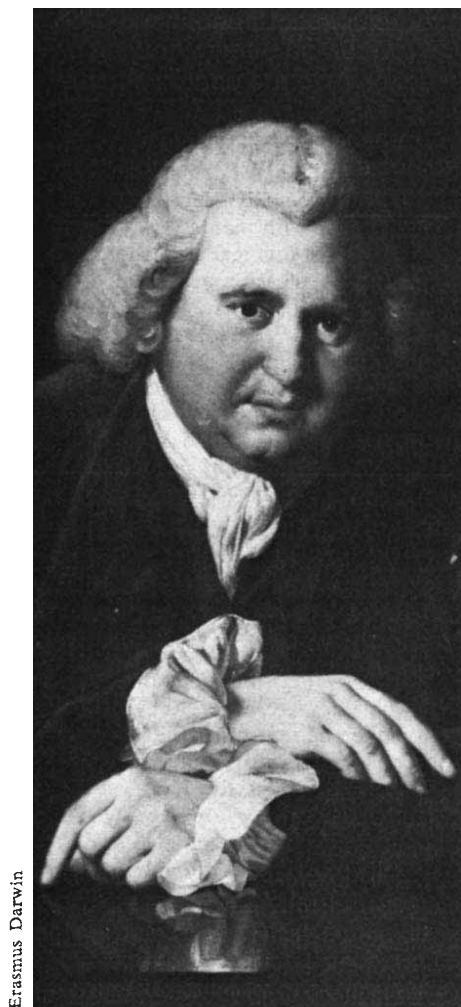
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Erasmus Darwin

Photo: Edward Leigh, Courtesy Master & Fellows, Darwin College, Cambridge

Genetic variation and selective forces

Kenneth Mather

Inheritance and Natural History. By R. J. Berry. Pp. 350. (Collins: London, 1977.) £6.50.

THE first book in the New Naturalist series was "Butterflies" by E. B. Ford in 1945. In it he spent about one-third of his space stressing the importance of genetic differences, of natural selection and of the numbers of individuals for our understanding of British butterflies as we see them in the wild. It is a tribute to the effort devoted to these aspects of the study of wild populations during the past 30 years, not least by Ford and his students, that the latest (number 61) in this series, written by R. J. Berry, is given over entirely to them. As the editors point out in their preface—unexpected as an addition of this kind may seem to a series dealing primarily with the British fauna and flora—such a book can do much to show us how our plants and animals have come to be as we see them today and how their changes and adaptations are still continuing. In his own preface the author describes his main aims as being "to show the ways in which inherited variation can help to explain the properties of natural populations". And he might have added—"and how the properties of these populations enhance our understanding of the ways in which genetic principles work out in the complex situations of the real world".

The book is of course written primarily for the naturalist, and although the familiar principles of genetics are set out in the early chapters they do not appear in the familiar order or with the familiar emphasis of the student's textbook. In fact, the emphasis from the start is on populations, their uniformities and heterogeneities, and the ways in which they reflect differences due to the ages of the individuals, seasonal influences and the direct action of the environment as well as those due to genetical variation. The basic structure of the genetic materials is described, as of course are the principles of Mendelian inheritance, of the interaction of genes in producing their effects, and of the ways in which one gene difference may affect many characters and one character be affected by many genes. Linkage is also mentioned, but its treatment is brief.

The second chapter takes us to the basic principles of population genetics, starting with the Hardy-Weinberg principle and its use in understanding the

genetic structure of populations. Departures from the Hardy-Weinberg equilibrium are considered and the disrupting agencies discussed. Small populations and the drift of their gene frequencies are dealt with at some length, and the consequences of the founder-effect are illustrated by reference to wild populations of voles. It might be objected that the genetic basis of the tooth character at issue in these voles is not clearly established, but it could be held that the detailed genetics is not essential for the naturalist to appreciate what is happening. Mutation, migration, non-random mating and natural selection come next, though fuller discussion of the last two is reserved for later chapters. The notion of genetic equilibria being reached by the opposing actions of these various agencies is also introduced. Chromosome variation, both numerical and structural, is described once again in terms of natural populations and their chromosome polymorphisms.

A chapter is devoted to modes and rates of reproduction and to the various devices that organisms have developed to control their frequencies of inbreeding and outbreeding. This inevitably draws heavily on plants, with their greater variety of breeding systems; here, one feels that Professor Berry is on less familiar ground. It will surprise plant geneticists to find apomixis described as including vegetative reproduction and also as invariably leading to the production of clones. It will also surprise them to find spurges accredited to the genus *Mercurialis*.

The chapter on natural selection covers not only the basic types of selection—stabilising and directional, frequency dependent, density dependent, and so on—but also illustrates both mechanisms and difficulties by reference to the spread of, for example, warfarin resistance and industrial melanism on the one hand, and to the problems of the *gens* in cuckoos and area effects in snails on the other. It is good to see here a reference, albeit a brief one, to the action of selection in domesticated animals and to the way that quantitative variation and its responses to selection are measured and analysed by breeders.

Having dealt with these basic phenomena of genetic variation, reproductive and mating systems, and natural selection in its many forms, Professor Berry turns to populations themselves, their structures and relationships to one another. He discusses gene flow, especially in clines, and the conservative features such as co-adaptation, linkage disequilibria and super-genes, which the genotypes of the individuals show. He considers the consequences of ecological as well as geographical separation and of barriers like the Tingwall Valley in Shetland, though curiously he makes virtually no mention of the remarkable unseen barrier that must be inferred to account for the abrupt

change in spotting of *Maniola jurtina* near the eastern border of Cornwall. A feature which lends great interest to these discussions is the summary of his own extensive work on field mice, voles, human skulls and, especially, the house mice of Skokholm—this last being the vehicle which he uses to draw together the ways he sees the various processes as acting in determining the properties of a population. And he concludes with a chapter entitled "Why be variable?" covering such matters as the so-called genetic load of a population, *r* strategies and *K* strategies in relation to the species' biology, the significance of neutral mutations, and the importance of niches in maintaining variability.

This book is not easy to summarise and assess. It should not be judged by the criteria of textbooks, for as the author points out in his preface it was not written as such. This, he adds, relieves it of the need to be as inclusive and balanced as a textbook, and indeed "the chapters that follow involve a selective and therefore idiosyncratic choice of topics and examples". In places, the choice has perhaps been a little over-idiosyncratic, as in the chapter on "Human Influences", which covers everything from Muller's CIB technique for measuring the frequency of mutation to sex-linked lethals in *Drosophila* and the genetic changes in stock animals during their domestication, to human races and eugenics. The book could indeed be criticised as somewhat haphazard in its arrangement and uneven in its treatment. And it is made irritating in places by minor slips and inaccuracies, though some at least of these may stem from a relative unfamiliarity with plants.

Yet, at least one reader of the book has derived both pleasure and profit from it. It is full of information and it clearly brings out the complexity of genetic variation on the one hand and of selective forces on the other. It touches on the action of selection in moulding the genetic system and, while recognising the supreme importance of natural selection in shaping populations, it emphasises the significance of discontinuities and founder effects in setting the range of variation on which selection can act. But above all it is the wealth of examples taken from natural populations and the author's clear enthusiasm for observing wild creatures and trying to understand why they are what they are, where they are, which will make one remember the book. It was written for naturalists many of whom will I believe find in it the ideas and interpretations that they are seeking. And I hope that at least some students will read it and profit by it, even though it is not the textbook from which their fundamental knowledge of genetics must come. □

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Race and intelligence

William J. Schull

The Race Bomb: Skin Color, Prejudice, and Intelligence. By Paul R. Ehrlich and S. Shirley Feldman. Pp. 256. (Quadrangle/New York Times Books: New York, 1977.) \$9.95.

The Race Bomb is still another attempt to redress the misconceptions and prejudices which surround the ostensible association of the colour of our skins with our intellectual abilities. Ehrlich and Feldman—the former a population biologist and the latter a psychologist—deplore the diversion of effort which attends the controversy over race and intelligence, but feel compelled apparently to add their ruminations to dispelling the myths and misunderstandings. In this, I wish them well but I am not sufficiently sanguine to believe much will be accomplished. Digenes was surely not searching for an honest man, but rather one dissuaded from the comforts of prejudices by the logic of the arguments of another.

Few of the uncommitted will be influenced by this book, for it is as frequently tendentious as intellectually compelling, and panders to the commercial. The latter is a serious charge, but how else is one to interpret a title so patently intended to scare. But is the book, in fact, tendentious? I offer the following evidence: We are treated, for example, to apocryphal remarks alleged to have been made in a Salisbury (Rhodesia) hotel to one of the authors, and to a series of self-serving statements of others, admittedly biased but of such antiquity (about 1890 and 1926) that the irrelevancies which follow such as “Can you guess x’s race (colour)?” hardly appeal to whatever rationality man may claim.

My disappointment with this book is not so much with the thrust of the arguments, with which I agree, nor the interpretation of the data which are cited and are generally sound, but rather the manner in which the arguments or interpretations are set out. Are statements made by Shockley or Jensen more or less trustworthy simply because they made them? If an argument is, indeed, specious or worthless, need it be so designated at the outset? Why shouldn’t the intelligent reader be allowed to reach these conclusions himself or herself? Or is it because the arguments are not so persuasive? In some instances, I suspect the latter. For example, on p129, the fact that illegitimate (German) children 5 to 13 yrs old, the offspring of Black and White Ameri-

can servicemen, did not differ in average intelligence is advanced as evidence of no racial differences in intelligence. The Armed Forces have had, however, and as far as I know, still have minimum standards of intellectual skills for enlistment. Thus, data of the kind cited can be used in the broader context only if the proportion of Blacks and Whites rejected are comparable. Or, the comparison of trans-racial adoptions with intraracial ones is clouded by the involvement of the parents—unlikely to be equal in the two instances—and a burden of historic guilt, real or imaginary, carried by many.

I think some of my disillusionment stems from the expectations which I brought to the reading of *The Race Bomb*. I feel betrayed; instead of the tightly reasoned series of arguments which I had anticipated, I got a sententious polemic. Can’t individuals presented with “the facts” be expected to reach sensible interpretations? Or are we all so ill-deserving of trust or so unintelligent that arguments must be labelled?

The Race Bomb is not without merit, nor do I so intend to suggest. The chapter on “Intelligence and Intelligence Testing” is an especially good, albeit brief, statement of the manner in which intelligence tests are constructed, administered and scored. Types of tests are described as well as what they measure and do not measure. Issues such as the stability and constancy of the IQ are addressed, and so is the matter of when a test result may be viewed as significant, even within the properly restricted limits of the principal aim (to predict school performance) of the test. This chapter is written without cant or hyperbole. Why couldn’t more of the others have followed suit? Some do, in part. Thus, for example, the chapter “Is IQ Inherited?” begins with a series of simple, but clear statements on the roles of man’s nature and nurture, the utility (or otherwise) of concepts such as heritability, and various types of human biological studies which may, granted certain assumptions which are often not satisfied, provide insight into the relative importance, under a given set of circumstances, of environmental and genetic influences. But it strays to personal issues; I cite the following quotation: “Those not interested in going further [into an account of Burts’ scientific peccadillos—*my addition*] might wish to ruminate on the idea of a society that awards knighthood for ‘science’ of the calibre of Burt’s”. This seems to me to be about as pertinent to an analysis of why discrimination and prejudice exist as to fault the Nobel Society because it has awarded prizes for the proof of the parity principle as well as its disproof. Each of us, and the societies of which we are members, can do no better than what reason leads us to with the information

at our disposal. How much can one be faulted for not doing what he or she doesn’t know. One may commit a stupid act, but is it morally reprehensible, or as an earlier era might designate—sinful? I’ve always been lead to believe that concupiscence was essential to a sinful act. Does this mean that I condone what has occurred and about which these authors are justifiably disturbed? Not at all! But if we are or at least purport to be a rational animal, shouldn’t we attempt to treat one another as such? How can one convince but not perjure?

This book will provoke many and make smug an equal or perhaps larger number. But will it persuade the “uncommitted”? I believe not; but it may urge others to form their own opinions. Read it. Some day, but apparently not soon, we may see a succinct, forceful, objective account of this controversy—an account which eschews arguments *ad hominem*, the same old “*dramatis personae*”, and the seemingly irresistible urge to act out once again what has become an initiation ritual into the fellowship of liberals. When this occurs I hope *Nature* gives me the opportunity to review it. I don’t revel in the role of curmudgeon. □

William J. Schull is Director of the Center for Demographic and Population Genetics and Professor of Population Genetics at the University of Texas, Health Science Center, Houston, Texas.

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Genetics and society

Harvey L. Levy

Genetics, Law, Social Policy. By Philip Reilly. Pp. 275. (Harvard University Press: Cambridge, Massachusetts and London, 1978). \$15; £10.50.

It is doubtful that Robert Guthrie could have anticipated the Pandora's box of legal and social issues that would be opened by genetics when he introduced the newborn screening test for phenylketonuria (PKU) in 1962. But the box did open and from it has poured some of the most difficult questions faced by society. Should testing for genetic disease be mandatory? What information should be transmitted by genetic counselling and how should such transmission occur? What are the relative responsibilities of parents and society in determining procreation and birth in certain genetic circumstances? Is genetic information to be held in absolute confidentiality or does the public welfare require numerous exceptions to this?

Within recent years several books that examine at least some of these subjects have been published. Among them are *Ethical, Social and Legal Dimensions of Screening for Human Genetic Disease* (edited by D. Bergsma), *Genetic Screening: Programs, Principles and Research* (by the Committee for the Study of Inborn Errors of Metabolism, National Academy of Sciences), and *Genetics and the Law* (edited by A. Milunsky and G. J. Annas). Only the latter attempted to include legal and social issues of genetics in a somewhat comprehensive manner rather than relating them specifically to screening. Although it was a valuable contribution, *Genetics and the Law* inevitably suffers from lack of focus and redundancy, those twin banes of all edited books.

I therefore read the publication announcement of Philip Reilly's *Genetics, Law, Social Policy* with a good deal of excitement. Mr Reilly is certainly highly qualified to write on this subject—being a lawyer, having studied genetics under Dr Margery Shaw at the University of Texas and having served a fellowship in the Program in Law, Science and Medicine at Yale Law School. For the past several years he has devoted virtually all of his professional time to obtaining information about genetic programmes and to examining these programmes.

In *Genetics, Law, Social Policy* Mr Reilly has produced a valuable and

interesting book. Although not truly comprehensive in the many genetic issues that confront law and society, the book does include in-depth discussions of some of the major issues, including genetic screening laws, the role of society and the individual in eugenic decisions, legal issues concerning genetic counselling and unconventional reproductive techniques, and the increasing storage and potential public availability of individual genetic data. In each of these areas Mr Reilly summarises the past and present states, isolates those elements that pertain to the law, and raises those unanswered questions that seem most pertinent to present and future practice.

The strength of the book lies in the chapters on laws that mandate screening. Here Mr Reilly reviews not only the laws themselves but the legal basis for such laws and the potential bases for their legal challenge. He clearly points out the tension that exists in such laws between the desire of society to protect the individual and the right of the individual (or his parental protector) to freely choose his or her destiny. The screening laws covering the newborn fare much better in this regard than do those that mandated sickle cell detection, the latter being soundly criticised. On balance, Mr Reilly has given a fair and comprehensive treatment of this subject.

There is particular value in the chapter on the future of genetic screening legislation, which Mr Reilly sees as on the increase and which needs to be carefully controlled and constructed. The chapter on eugenic policies as they confront personal freedoms contains less substantial matter than those on genetic screening laws but is nevertheless very interesting and establishes a basis of information for future such considerations. The same can be said for the last section which contains the chapters on genetic counselling, new reproduction technologies, and genetic data. Most of the discussion in this section concerns somewhat brief descriptions of the current situation and considerations of probable future problems.

The book is weakest in the first chapter which attempts to summarise current concepts of human genetic disease. The geneticist will certainly not need to read this and the non-geneticist would be advised to look elsewhere for a more accurate introduction.

Despite this deficiency the book is highly recommended as an introductory reference work for geneticists and all others interested in current and future relationships between genetics and society. □

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Genetic scaremongering

David Baltimore

Playing God: Genetic Engineering and the Manipulation of Life. By June Goodfield. Pp. 218. (Random House: New York; Hutchinson: London, 1977.) \$8.95; £5.95. *Improving on Nature: The Brave New World of Genetic Engineering.* By Robert Cooke. (Quadrangle/New York Times Books: New York, 1977.) \$12.50.

THE techniques of recombinant DNA have produced an unpredicted bonanza: a new field of research employing people who write about it, think about it, and collect its artifacts. The politics and sociology of the not yet existent enterprise of genetic engineering, and the moral and ethical aspects of its vaguely conceivable possibilities are the basis for books, articles, lectures, symposia, courses, journalism, philosophy and all manner of academic practice.

Is it all worthwhile? One thing is sure, the impact of modern biology on society will have been thoroughly considered long before it is a reality. But will this

consideration reduce the ill-effects of new technologies and improve the chances that we will integrate future methodologies into our scheme of life with less trauma than in the past? It seems doubtful, because one of the facts of scientific life is unpredictability. What you think will be easy is often hard or impossible; what you never conceived of falls into your lap. So it is hard to prepare society for the impact of science when the form of that impact is so difficult to prophesy.

June Goodfield and Robert Cooke in their recent books do a service even if their detailed worries may be wide of the mark. They prepare their readers for thinking about the future. They introduce the language of modern biology; they describe advances to date; they raise questions to think about; and they tell some interesting stories. But their books suffer from being too superficial, not always accurate and not very surprising.

Cooke's book—in the breezy style you might expect from a journalist (he is science editor for the Boston Globe)—introduces us to the many areas in which genetic manipulation is being used or may be used in the future. After describing recent scientific advances in cell biology, genetics and molecular biology, he then catalogues the many areas of modern life that could be affected by new abilities of

genetic manipulation. In the most interesting section of his book he shows how medicine, agriculture and industry could all be revolutionised by being able to apply new methods of genetic alteration. Finally, he discusses safety issues relating to recombinant DNA and ethical issues relating to genetic engineering.

The best parts of this book are marred by sensationalism. "Look soon", he tells us, "for a biologist somewhere to announce he's finally built the first man-made mouse". Elsewhere, he suggests that "Future genetic treatment for emphysema will probably involve . . . growing up a whole new set of lungs in tissue culture or even the implantation of healthy new genes". His inability to separate science and science fiction is hardly a problem unique to him but seriously compromises the utility of his book.

Cooke also mixes up so completely the safety aspects of recombinant DNA methods with worries about genetic engineering that he badly muddies an already murky discussion. Goodfield does a much better job of keeping the two areas separate. Even though recombinant DNA methods are likely to speed the development of ways to modify gene expression and inheritance, the questions posed about the safety of recombinant DNA research procedures are independent of ones about genetic engineering.

Cooke ends by pushing Sinsheimer's view that recombinant DNA work should be relegated to a few isolated laboratories. Nowhere, however, does Cooke demonstrate that there are any hazards to recombinant DNA work; he merely accepts quotable remarks of some of the fearmongers who would rather listen to their own imaginations than to think through the questions rationally.

Goodfield's book is of a different sort: it is more erudite and is often a personal odyssey. She tells us about her own attempt to do recombinant DNA work, and about her discussions with various scientists of their reactions to genetic engineering possibilities. Much of the book is an interesting historical analysis of the relationship of scientists to society. She brings us back to the attitudes surrounding the founding of the British Association for the Advancement of Science in the mid-nineteenth century as she develops the very important question of whether science should be free of societal restraints or should be tightly constrained by public accountability.

The major problem with her discussion of the potential impact of biologists' actions on society is her deep trust of her own "humaneness". She believes that this amorphous concept provides a basis for establishing a value system that can guide future applications of technology. She rests heavily on the fashionable notion of "natural" (that is, predating

later human intervention) but adds little of precision to the debates about how to mould technology to humane ends. She greatly fears that man is about to be grossly altered by new genetic technologies but her flowery prose and deeply-felt exhortations rather obscure than illuminate the problems.

She understands the science of molecular biology less well than Cooke and she often makes errors, but they matter little because her aim does not seem didactic. She asks, for instance, whether there is or should be an implied contract between scientists and society as there is for doctors and lawyers. Her insistence on asking what constitutes the responsibility of scientists to society raises the crucial issue of how much science is its own discipline—responding only to the inner dynamics of the search for truth—and how much it is a response to societal needs. When science is seen as the servant of society, its priorities are likely to be very different from when it is seen as an aspect of man's culture. A problem she does not raise is how to maintain the freedom that nurtures scientific creativity and still make science responsive to direction from society.

Molecular biology grew up as a pure science with only tenuous connections to real-world problems, but recombinant DNA methods and the debate they have engendered have, as Goodfield rightly points out, signalled a change in the status of the field. Now that molecular biology seems to be relevant to practical concerns, it is going to have to answer to society in a more direct way than before. US Senator Edward Kennedy picked up this perception a number of years ago and has struggled to frame legislation that reflects it. While many believe that he has not been careful enough to safeguard the independence that science needs if it is to continue to make qualitatively new contributions, he has rightly seen that modern biology must meet new demands commensurate with its new powers.

I wish that these had been better books. Cooke could have taught us more, Goodfield could have allowed her intellect to probe the questions she raises rather than taking refuge in emotion. Both books would have improved if the authors had avoided the rhetorical trick of saying that scientists are "playing God". It is hard to know what the authors think that means but it allows them to avoid the difficult job of understanding the play of evolutionary forces that has brought the natural world to its present state. If they had understood the power and magnificence of the forces that moulded the present, rather than hiding their ignorance in the vagueness of theology, they could have better served their subject. □

David Baltimore is Professor of Microbiology at the Massachusetts Institute of Technology, and a member of the original Berg committee.

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Evolution of human intelligence

N. J. Mackintosh

The Dragons of Eden: Speculations on the Evolution of Human Intelligence. By Carl Sagan. Pp. 263. (Hodder and Stoughton; London, 1978.) £5.95.

CARL SAGAN is a professor of astronomy at Cornell and author or editor of several books such as *The Cosmic Connection: An Extraterrestrial Perspective*, and *Communication with Extraterrestrial Intelligence*. In *The Dragons of Eden*, he turns his attention to our own planet, and has written a personal, speculative account (his words, not mine) of the evolution of human intelligence. The new book has had great success in the United States (Random House: New York, 1977, \$8.95; Ballantine: New York, 1978, paperback \$2.25), and arrives here amidst much publicity. The publishers' blurb promises us "a breathtaking overview" containing

"quite literally mind-blowing revelations" (literally?) about "how human beings evolved, brains and genes together".

The style is breathless, if not breath-taking. "Somewhere in the steaming jungles of the Carboniferous Period there emerged an organism that for the first time in the history of the world had more information in its brain than in its genes". Or "The R-complex [an entity of which more anon] is functioning in the dreams of humans; the dragons can be heard, hissing and rasping, and the dinosaurs thunder still". Sagan is much given to wonder as well as to purple passages. He wonders whether "the ritual aspects of many psychotic illnesses" and "the frequent ritualistic behaviour in young children" (what young children has he been watching?) represent a failure of neocortical control. And noting that australopithecines were only three-and-a-half feet tall, "I wonder," he tells us, "whether our myths about gnomes, trolls, giants and dwarfs could possibly be a genetic or cultural memory of those times".

This, then, is a popular book, written by a non-specialist straying outside his academic fold. If it has some of the virtues of its kind, being reasonably free of jargon, easy going, occasionally interesting, and often nice to look at, this is amply compensated for by its vices. It is inaccurate, full of fanciful and unilluminating analogies, infuriatingly unsystematic, and skims hither and yon over the surface of the subject, unerringly concentrating on the superficial and misleading at the expense of the important or significant. That it should have had such success is a sad commentary on the power of publicity.

The book is well produced and easy on the eye. There are numerous illustrations of tangential relevance to the text. Sagan's favourite artist seems to be Escher, but others who have painted or sculpted serpents and dragons are not neglected. The text itself contains some quaint, if not particularly useful, snippets of information. I did not know, for example, that Lord Byron's brain was twice the size of Anatole France's, nor that Pliny had supposed the ostrich to be the result of a cross between a giraffe and a gnat (can he really have thought that?). Nor had I come across the notion that our mistrust of left-handedness (*sinister*, *gauche*), and preference for using the right hand in greeting might have arisen from our ancestors' habit of using the left hand "for personal hygiene after defecation".

But these are not great virtues. The inaccuracies alone suffice to negate them, especially when they come as thick and fast as in this single sentence: "A fish has a notochord or spinal cord, which it shares with even humbler invertebrates". And all too often the snippets of information distract rather than inform. Does it

help one understand what an average brain volume of 1375 ml means to be told that 1 ml is about the volume of an adult human navel? At times, indeed, the book degenerates into a series of random odds and ends, strung together without rhyme or reason. One chapter, for example, entitled "Tales of Dim Eden", starts with some information about brain waves, shows us the electroencephalogram patterns characteristic of waking, sleeping and dreaming, and provides some speculations about the functions of sleep. We then move on to a discussion of the brain size of dinosaurs, some speculations on the causes of their extinction, and the suggestion that the evolutionary conflict between mammals and reptiles is mirrored in the story of the serpent in Eden. We read about the Komodo dragon of Indonesia, "a predator exhibiting a chilly fixity of purpose", and are told that stories of dragons are common in all mythology and folk tales. We then return to dreams and their functions, are asked to believe that "major evolution beyond the reptiles has been accompanied by and perhaps requires dreams", and are finally informed that the true function of dreams is to allow free play to the ancient, reptilian parts of our brain (the R complex). Notwithstanding which, dreams about falling are said to reflect our arboreal origins as primates, and those about snakes our mammalian fear of reptiles.

If he perseveres to the end, therefore, the innocent reader will have sipped on a random array of information, alternately entertaining and bizarre, will have felt his flesh creep with stories of dragons, and will conclude with some mildly uplifting exhortations to trust to reason and science rather than the occult or irrational—a nice touch this in a book so profoundly unscientific. Will he have learned anything about the evolution and nature of human intelligence? Not much. It is a pity. It is an important and fascinating topic. We all, no doubt, want to know more about ourselves. What manner of animal are we and where do we come from? What makes us different from other animals and what do we mean when we say we are more intelligent than them? But we must temper our fascination with some respect for hard evidence, and therein lies the problem, for in the nature of the case these are questions about which it is easier to speculate than provide such evidence. From palaeontology we know that the evolution of the human brain followed rather than preceded the development of walking on two legs, and that early hominids made tools and ate meat. We can thus speculate that human intelligence developed in response to tool making and cooperative hunting, but the inference is by no means as plausible as is popularly supposed. One need only, for example, look at chimpanzees to see that walking on one's hind legs is hardly a

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necessary precursor to using one's hands to manipulate or fashion implements. And what is the relationship between the brain and intelligence anyway? That there is *some* relationship between the size of one and the degree of the other seems probable. But it is surely not as close as is sometimes assumed, and one suspects that the use of brain size as an index of an animal's intelligence is more a consequence of the lack of any better measure rather than a reflection of its intrinsic worth. Sagan is by turns sensible and rash on this topic. He starts cautiously, but in a few pages one reads, for example, that "the evidence is quite compelling that mammals are indeed systematically more intelligent than reptiles". The only evidence mentioned (unsurprisingly, as there is no other available) is that the ratio of brain weight to body weight in mammals is significantly higher than in reptiles, but this by itself proves nothing.

In common with others who should know better, Sagan is prone to naive neurologising. The central thesis of his book, indeed, is a classic example of this tendency. Because parts of the human brain are supposedly homologous to parts of the brain of reptiles, Sagan jumps to the unwarranted conclusion that they continue to perform the same, reptilian functions. And a sinister set of functions they are: aggression, territoriality, hierarchy, and ritual. Of these, his favourite is ritual—perhaps because of the alliterative delights of talking of the ritualistic, reptilian functions of the R complex. At any rate, the desire to dress up, the belief in sacred rites, and the compulsion to obey mysterious laws, are all a consequence of our reptilian ancestry. Lip service is paid to our ability to overcome these primitive impulses (through neocortical control), but the dark hint is there. Not that it lacks a lighter side: I particularly liked the "reptilian ritualization of the educational process".

The brain is a physical entity, and those who need to see a piece of machinery before they can accept that it performs certain functions will always be tempted by facile explanations of psychological process in terms of neurological structure. As neither psychological process nor behaviour leave fossil evidence, the temptation is all the more irresistible when we ask questions about the evolution of behaviour. Brains themselves, of course, do not fossilise, but skulls do; from fossil skulls we can reconstruct endocasts, and from these we can make more or less informed guesses about the size of the brain of some extinct ancestor of man. It is important to realise how extraordinarily little this will have told us about his behaviour. Perhaps we would do better to study the behaviour of those living species most closely related to man. Sagan does not neglect this alternative,

and takes us on a quick tour of some of the more widely popularised features of monkey and chimpanzee life. We learn of Japanese macaques learning to unwrap caramels and sifting wheat from sand, and transmitting these new habits to other members of the troop; of chimpanzees fishing for termites in East Africa, and learning sign language in Nevada. Every example is treated with bated breath. Together, they suggest to Sagan that within a few generations we may see a community of chimpanzees from which "might emerge the memoirs of the natural history and mental life of a chimpanzee, published in English or Japanese (with perhaps an 'as told to' after the by-line)". Whether or not Sagan wishes us to take this possibility seriously, it remains entirely typical that he nowhere considers just what intellectual or cognitive capacities are implied by the feats over which he enthuses. Learning to fish for termites, it seems, is very much a matter of trial and error, there being no way to predict from rational considerations what sort of twig is best adapted to the task, nor how to twiddle it around, nor even where to put it, and young chimpanzees learn these skills largely by careful observation of their elders. Neither trial and error learning nor social imitation are particularly impressive examples of higher intellectual processes, and neither, of course, is confined to primates. Although Sagan tells us how marvellous it is that chimpanzees can be taught to communicate by a sort of gestural language, he does not discuss what is involved in this behaviour, nor how it differs from human language; nor does he even mention the work of David Premack, the one investigator of chimpanzee 'language' who has attempted to understand some of the cognitive processes and skills underlying it.

Characteristically, it is the fanciful and arresting implication that catches Sagan's attention. Thus, "if chimpanzees have consciousness, if they are capable of abstractions, do they not have what until now has been described as 'human rights'? How smart does a chimpanzee have to be before killing him constitutes murder?". That is a question worth considering, but it seems only fair to conclude with a more typical Saganism. If "there are non-human primates so close to the edge of language, so willing to learn, so entirely competent in its use and inventive in its application . . . why are they all on the edge? Why are there no non-human primates with an *existing* complex gestural language?" The answer? It is because "humans have systematically exterminated those other primates who displayed signs of intelligence". □

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New books from Blackwell

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Edited by H.G. Reading. Summer 1978. 400 pages, 280 illustrations. Cloth about £18.00, paper about £11.60

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Edited by S.D. Gerking. June 1978. 500 pages, 60 illustrations. About £21.00

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Edited by P.R. Scott and A. Bainbridge. Summer 1978. 256 pages, 50 illustrations. About £9.00

This book discusses the dispersal of plant pathogens, monitoring of diseases and their environment, mathematical modelling of epidemics, forecasting of disease outbreaks, and ways in which an understanding of epidemiology can lead to disease control.

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Discussion on interactionism

O. L. Zangwill

The Self and Its Brain: An Argument for Interactionism. By Karl R. Popper and John C. Eccles. Pp. 597. (Springer: Berlin and New York, 1977.) DM39; \$17.20; £9.40.

It is rare to find an experimental scientist whose ideas of scientific method have been decisively influenced by a philosopher. But Sir John Eccles has never concealed his indebtedness to Sir Karl Popper and the two men have evidently become firm friends. In September 1974, they were invited by the Rockefeller Foundation to spend a month at the Bellagio Centre on Lake Como, where they began to plan this book. In a series of discussions, they exchanged views on a variety of theoretical issues, not least that of consciousness, the mind-body problem, and the implications of their views for the biological sciences. They also began their respective contributions to the present book.

The book contains three main sections. Part I, written by Popper, deals primarily with philosophical and historical issues relating to cultural evolution, the mind-body problem and the biological approach to human knowledge. Part II, by Eccles, provides a conspectus of contemporary neurophysiological work on the brain as seen from the standpoint of one who believes in its direct interaction with the mind. Part III contains the original dialogues between the two authors which took place at the outset and which have been edited only in so far as is necessary to avoid duplication of material included in the main text. They may be read either in conjunction with the latter or as marginal comment on the entire enterprise.

Most biologists steer clear of the mind-body problem in their daily work, though many approach the problems of behaviour from a materialist standpoint and are apt to express strongly reductionist views. Both Eccles and Popper are implacably opposed to such a standpoint. They believe implicitly in the existence of mind as an independent entity and reject both psychophysical parallelism and the so-called identity theory, according to which mind and brain reflect different aspects of the same reality. Interactionism, on the other hand, according to which the mind is capable of exercising

a direct effect on the machinery of the brain, they regard as the only acceptable solution to the mind-body problem. Yet for all its commonsense appeal, scientists have always been most wary of such a solution, and in spite of its advocacy by two such very distinguished men it is unlikely to gain in popularity. Nevertheless, it is impossible to remain unimpressed by the authors' determination to face up to some of the most daunting problems which the biological and human sciences are called upon to confront. Their discussion makes plain that empirical issues can never be wholly divorced from philosophical and perhaps even from ethical considerations.

Not being a philosopher, the present reviewer proposes to concentrate on Eccles' contribution. In so limiting himself, he certainly implies no disrespect to Popper, whose intellectual standing is world-famous and whose lucid exposition of thorny problems in the present book can be followed by any scientist, even though he may be wholly innocent of education in philosophy. Indeed, his critique of materialism and historical comments on the mind-body problem will be of the greatest interest and value to the historian of science.

Sir John Eccles is a sometime pupil and colleague of Sherrington and seems to have been much influenced by the views on mind and matter expressed relatively late in life. It will be recalled that in his early work on reflex integration, Sherrington seemed to be confident that the principles that had served him so well in elucidating the activities of the spinal cord would equally well find application to our understanding of the nervous system as a whole. Just as the fractional activities of the body are integrated at the spinal level, so was the brain conceived to

integrate the activities of the whole organism in its adaptive relationships with the environment. Yet as he grew older, Sherrington clearly became unconvinced of the adequacy of such a view. "We are partly reflex," he wrote "and partly not". That part which is not reflex, Sherrington conceived to be in liaison with the mind, envisaged as an independent non-physical entity. It is this commitment to dualism that informs all Eccles' more recent writings on philosophical topics. But he seems to go somewhat beyond dualism in postulating not only that the mind is in liaison with the brain but also that it interacts with it in generating behaviour. Indeed this belief sets the tone of his entire contribution to this volume.

How does Eccles develop his theme? First, he gives us a lucid account of the anatomy of the cerebral cortex, conceived on the one hand as a physical system and on the other as the organ of liaison with the mind. In the latter connection, he directs special attention to the columnar or modular organisation of groups of cortical cells on which emphasis has been placed by Szentagothai and others, seeing in these cell-groups "linked together in mutual connectivities" a likely focus for interaction with mental events. He then proceeds to outline what is more or less reliably known from experimental physiology about the mechanisms of perception and voluntary movement. As might be anticipated, he places special emphasis on aspects of their study that might seem to have special relevance to mental activity—for example, Libet's work on discrepancies in the timing of electrical activity in the cortex and the onset of conscious sensation or Kornhuber's work on the cortical potentials generated before the carrying out of a voluntary action. Interesting and important as these observations may be, it does not follow that they require an explanation from the interactionist point of view. They are equally consistent with parallelism or identity theory.

Secondly, Eccles devotes several chapters to neuropsychological studies of the effects of focal brain lesions. He reviews the patterns of deficit resulting from circumscribed lesions of one or other hemisphere, in the course of which he makes abundantly clear that equivalent lesions of the two hemispheres by no means necessarily produce equivalent effects. Language disorders, for instance, are linked almost without exception with lesions of the left hemisphere, whereas certain visual and spatial disabilities are linked, if somewhat less exclusively, with lesions of the right hemisphere. As we shall see, however, he nevertheless maintains that liaison with consciousness is



Sir John Eccles

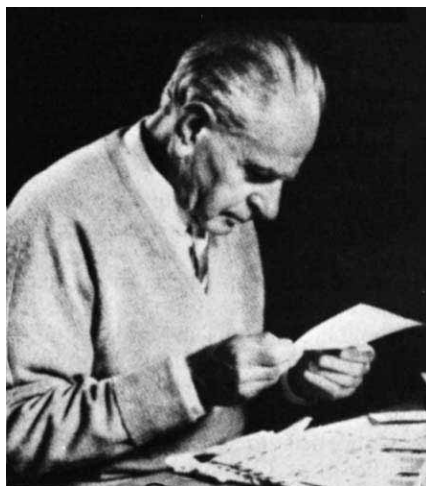
essentially an activity of the normally dominant left hemisphere. Although it is good to see work such as that of Brenda Milner, of Montreal, presented alongside the findings of traditional neurophysiology, Eccles is perhaps a little too prone to interpret evidence regarding localisation of psychological deficit as evidence in favour of localisation of psychological function. At the same time, he is surely on strong ground in advocating that sophisticated anatomical and physiological study of areas of the cortex that seem to be especially relevant to psychological deficit is urgently required. Quite irrespective of what views one may hold on the mind-body relationship this would seem to be the only way in which to gain knowledge of how the brain actually works in generating higher-order psychological activity.

Thirdly, Eccles devotes much space to the problem of consciousness and its relationship to the cerebral hemispheres. Here he draws heavily on the experiments of Roger Sperry and his co-workers on the psychological capacities of patients who have undergone complete section of the cerebral commissures for the relief of severe and intractable epilepsy. These experiments have led Sperry to the view that those who have sustained this operation can be shown to possess a certain duplication in their conscious activities. Although the main findings of Sperry's work are clearly and fairly described, it is evident that Eccles is highly critical of Sperry's interpretation in terms of duplex consciousness. Indeed, the problem of consciousness and its relationship to the cerebral hemispheres is perhaps the most challenging issue in neuropsychology today.

What are the facts of the case? Firstly, it cannot be denied that striking disconnections between the two hemispheres can be demonstrated if sensory input, visual or somatosensory, is restricted to one or other hemisphere alone. In such a case, there would be virtually no transfer to the opposite hemisphere. Each hemisphere can therefore act as an independent processor of information. Secondly, the two hemispheres differ strikingly in their capacity for verbal communication. Whereas the dominant (left) hemisphere can communicate freely in speech or writing, the minor (right) hemisphere, while possessing appreciable powers of verbal comprehension, is to all intents and purposes mute. Nevertheless, this hemisphere is demonstrably capable of response indicating higher-order capacity for perception, classification and abstraction. It is indeed actually superior to the dominant hemisphere in the execution of certain types of intellectual skill, particularly those involving visual and

spatial analysis and judgement. Not without reason, therefore, does Sperry ascribe intelligence and conscious awareness to the disconnected minor hemisphere. Indeed there is evidence in some of Sperry's experiments of concurrent perceptual activities being set up in the two hemispheres without mutual awareness or interaction between them.

Although there is no disagreement about these facts, Eccles gives them a completely different interpretation. In his Eddington Lecture, delivered in Cambridge in 1965, he stated categorically that the right hemisphere is to be viewed as a computer, capable of complex acts of discrimination and learning though totally devoid of conscious experience. Eccles further denied that



Sir Karl Popper

it was capable of initiating volitional activity, though this contention has not been sustained by Sperry's more recent work. Although Eccles has restated his objections to Sperry's view in several later books and papers, there are now indications that his position has of late become somewhat less extreme. In *The Self and Its Brain*, he fully concedes that the right hemisphere displays intelligent reactions, which he seems to have denied to computers, and that it has many sophisticated skills, particularly in the spatial and auditory domain. He further concedes that in certain adult patients who have sustained total excision of the left cerebral hemisphere on account of malignant brain tumour there seems to be some kind of residual consciousness and some very primitive linguistic activity. The present reviewer has had an opportunity to work with one such patient and can testify that, although severely disabled, in any ordinary sense of the term this man was fully conscious.

In the present book, it seems that Eccles now wishes to deny to the minor hemisphere not so much conscious experience *per se* as self-consciousness. By this, he means an awareness of personal identity and the continuity of

individual experience. Self-consciousness involves, too, such higher levels of abstract thought as are made possible by language and related forms of symbolism. As Sperry has always regarded the dominant hemisphere as being primarily concerned with language and the unified control of bodily action, the gap between his views and those of Eccles would now seem to have grown appreciably narrower. But even so, it is difficult to regard self-consciousness in the individual whose commissures are intact as in exclusive liaison with the dominant hemisphere, or to suppose that the left hemisphere alone possesses mechanisms exclusively adapted to the elaboration of high-level conscious experience.

Do the two authors agree about consciousness and the cerebral hemispheres? Certainly Popper accepts the self-conscious mind as an entity in its own right and as a distinctively human product of evolutionary advance. As a philosopher, he is naturally guarded in what he says about the brain but he certainly agrees with Eccles that the disconnected minor hemisphere is cut off from full self-consciousness. At the same time he considers that "... its achievements are so high that we have to attribute to it not only memory, which is a kind of prerequisite of consciousness, but a certain degree of creativeness as well". Indeed he confesses that he has been more or less convinced of Sperry's conjectures regarding consciousness and the minor hemisphere by the evidence which he has adduced as to its capacity for logical thinking as exhibited on non-verbal intelligence tests. At the same time, he has no doubt that it is language and the control of bodily action that endow the self-conscious mind with its distinctively human attributes.

Though biologists with a taste for philosophical speculation will find much to interest them in this book, not least Popper's brilliant historical comments on the mind-body problem and his spirited critique of materialism, they may well consider his defence of interactionism, his advocacy of emergent evolution and his curt dismissal of artificial intelligence, as out of key with contemporary scientific thinking. Even if Popper is right in supposing that no-one will ever build a computer with conscious experience, it does not follow that Kenneth Craik was necessarily wrong in his belief that machines embodying automatic control principles might well arise in the course of nature in sufficiently complex systems. One such system might well be the human brain. □

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Gunning for reductionism

Colin Blakemore

The Purposive Brain. By Ragnar Granit. Pp. 244. (MIT Press: Cambridge, Massachusetts and London, 1977.) \$12.50; £8.75.

IN this short book Ragnar Granit brings together two subjects on which he himself has worked—the visual system and the motor system—as the basis of a general monograph about brain function and as the vehicle to express an individual philosophy. His treatment of these two areas is business-like and straightforward, though even here his style is idiosyncratic and his personal opinions are clearly expressed.

In his treatment of vision Granit introduces both the concept of “feature-extraction” by neurones that are highly selective for certain “trigger features” in the retinal image, and a seemingly contrary hypothesis that cells in the visual cortex are tuned to different spatial frequency components in the complex distribution of retinal illumination, and thus may be involved in some sort of primitive Fourier analysis of the spatial pattern of light on the retina. Though he declares that the detection of “change, contours, and movement” (classical trigger features) is an essential part of visual analysis, he is, nevertheless, enthusiastic about the parsimony of the spatial frequency hypothesis and emerges on the side of the frequency freaks rather than the feature creatures.

The section on the motor system starts with the mechanism of the synapse, the function of reflexes and the role of muscle spindles in the control of muscle length. It continues with the hierarchical central control of movement, from the integrative function of the spinal motoneurone up to the topographical distribution and properties of upper motoneurons in the motor cortex, and the possible function of regions of the parietal lobe in the building of body image and in the operation of voluntary movement within external space.

All this is tackled with authority and vigour, and with a vitality that anyone who has had the pleasure of meeting Granit will instantly recognise. Ragnar Granit already has his place in the history of brain research. He was awarded the Nobel Prize in 1967 (together with Hartline and Wald) for his work on the retina; but, as Sir John Eccles noted at the time, he could equally have won it for his pioneering study of motor control.

He has a special place in the hearts of neurophysiologists because it was Granit and Svaetichin who, in the late 1930s, developed the techniques for recording from nerve cells with an extracellular micro-electrode: this step alone was revolutionary.

In the first five chapters, and in the last one, Granit expounds a personal philosophy of science: I suspect that his book will be read mainly for this, rather than for the more straightforward sections on brain function. Granit is gunning for reductionism and is not ashamed to call himself a teleologist. Physiology, he says, is “applied teleology”, and the teleological approach is especially valuable in brain research to predict whether the purpose of any particular piece of brain machinery “would either be well or badly realised by one or several of a number of alternative hypotheses or models”. Purpose, as the title of the book implies, is the central theme of Granit’s discussion of the scientific method, the nature of understanding and, especially, the mechanism of evolution.

The brain is undeniably a purposeful machine, and the more advanced the species the more complex (even devious in the case of man) the purpose that its brain can pursue. But the richness of purpose in animal behaviour leads Granit actually to doubt the adequacy of genetic theory to account for the evolutionary emergence of purposiveness. He writes: “I am resigned to an attitude of mild scepticism as to the completeness of its coverage of matters requiring explanations”. Consciousness is viewed as an “emergent novelty”, the pinnacle of a hierarchy of purposiveness. Conscious-

ness is said to be “the perfect instrument for realising adaptability” (but is there really a dependent relationship here: would a highly adaptable animal have to be conscious?).

Granit’s “scepticism” about simple evolutionary ideas leads him to the brink of a belief that may lose him some of his readers. If natural behaviour is so full of purpose, he says, natural selection must have purpose too. He declares himself quite satisfied with applying the term “purposiveness” to evolution itself. But here his logic is not transparent: undeniable adaptation of each animal to its environment is surely not evidence for some kind of grand design in the mechanism for the emergence of species. To be sure, there is a frustrating credibility gap between the elegant examples of selection of simple characteristics and the monumental complexity of the human brain; but most biologists cling to the unifying hypothesis of “chance and necessity” and are unwilling to admit that it cannot cope with the mystery of mind. Granit surely gives a clue to this apparently intractable problem when he points out that adaptability (a kind of freedom from the constraints of innately coded instructions), not adaptation to a single environment, is “the glorious climax of evolution”. The richness of human behaviour is essentially epigenetic: what we have our genes to thank for is the generalised capacity to seek purpose in our actions, not the impossible feat of specifying everything we can do. □

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Philosophy of ignorance

P. B. Medawar

The Encyclopaedia of Ignorance. Vol. 2: Life Sciences and Earth Sciences. Edited by Ronald Duncan and Miranda Weston-Smith. Pp. 433. (Pergamon: Oxford, 1977.) Paperback £3.50. Hardback combined edition with Vol. 1 on the Physical Sciences £10.

IF, as the Philosophy of Induction invites us to believe, the sciences begin as great heaps of factual information which are “processed” according to a programme of ratiocination known as “the scientific method”, then it is difficult to see why we should ever be ignorant of anything that we want to know or understand; if the inductive scheme were true, there could be only two reasons for our being so: either (a) our senses are so obfuscated by

prejudice or sin that we cannot observe Nature aright; or (b) we are not performing “the scientific method” correctly. Possibility (a) was taken very seriously by the founding fathers of modern science, many of whom, doctrinary Puritans, believed that after the Fall, human beings lost the innocent perceptiveness that made it possible for them to recognise the Truth that Nature was simply waiting to divulge to those who would observe her intently and without preconceived ideas. Certainly this was John Milton’s view, for whom the purpose of learning was “To repair the ruins of our first parents”. Possibility (b) would carry some weight if the avowal of ignorance were a confession of failure by scientists whose past inability to make head or tail of Nature was already evidence enough that they did not really know or understand the scientific method—an explanation clearly falsified by the fact that the scientists who are most conscious of their imperfections and of the boundaries of their present understanding are often the most brilliant and empirically the most successful. Many of the authors of this symposium are

experienced and capable scientists: if there is such a thing as "the scientific method", they know it. They also know how to write clearly and simply.

Ignorance or a failure of understanding is, in my opinion, usually due to a failure of imagination: if we cannot even imagine what the truth might be—cannot make even a wild guess at an explanation that would accommodate our findings—then we are scientifically sterile, for every advance in science begins life as an imaginative preconception of what the truth might be.

It is, of course, possible that some scientific problems are insoluble; Sir John Kendrew, in his introduction to the symposium, shares with J. C. Eccles and K. R. Popper (in *The Self and Its Brain*, Springer: Berlin, 1977) the view that the body/mind relationship may be a case in point, having regard to the fact that the mind is both the subject of the investigation and the means by which it is prosecuted. This form of insolubility, I guess, is better described as "incompleatability"—just as (to borrow an example of Popper's) a portrait of a studio which includes the painter himself sitting at his easel with canvas in front of him is essentially incompletable (Gödel has made a cognate point in the context of mathematical logic).

This book, which embodies a good idea admirably carried out, will delight a biologically literate readership, for it is entertaining, informative and deeply thought-provoking—an entertaining and salutary graduation prize for a newly fledged biologist or botanist.

From the standpoint of the history of ideas, it is profoundly interesting to see how many of these essays have to do with evolution. The evolutionary conundrums put by Maynard Smith arise out of the innocent-sounding question whether a sustained doubling of mutation rate in the Mesozoic would have greatly speeded up, or not have had much effect on, the extinction of dinosaurs and the origin of mammals. Unfortunately, the factors that determine mutation rate and those that lead to extinction are not yet fully understood.

At a time when social evolutionism has tended to become almost as much of a philosophical nuisance as social Darwinism was in the days of Haeckel and Galton, Maynard Smith's views on altruism are particularly salutary. The analysis of altruism turns upon a clear recognition of the fact that it is the genes that reproduce while organisms are merely the agents that make it possible for them to do so: altruism is made possible by a state of affairs in which a favoured gene is propagated by proxy. Maynard Smith recalls Haldane's famous epigram that he was prepared to lay down his life for two brothers or eight cousins, and explains its significance thus: "A gene which reduces the probability of survival of an individual

carrying it but produces a corresponding increase in the fertility or probability of surviving relatives can increase in frequency. Thus, even the supreme form of altruism envisaged by Haldane could evolve by what we nowadays think of as orthodox mechanisms. The form of selection at work here is known as "kin" selection—Maynard Smith suggests nepotistic selection and adds "that kin selection affects evolution would be admitted by everyone who understands the laws of inheritance, and who is capable of logical thought. But it is much harder to say whether the process has been an important one. Opinions vary from those who regard it as, at the very most, relevant to understanding a few special cases, to those who see it as a major key to the understanding of normal societies including our own".

I was especially glad that Maynard Smith turned next to drift, the subject of the "most protracted debate in evolutionary theory". Right from the outset Professor Fisher's resentful opposition denied the idea a fair hearing. "Drift" is a change in the genetic make-up of a population, especially a small population, brought about by the fact that the gametes which unite to form the next generation cannot be an exactly proportionate sample of the population that gave rise to them. Here, too, we have a case similar to that of altruism: the problem is not whether or not the phenomenon occurs—it surely does, or at all events cannot be said not to—but rather how important the phenomenon is in bringing about evolutionary changes. In any event, all parties agree that selection is the principal agent of adaptation, which is "the most striking feature of the living world."

Where Maynard Smith writes of the scientific imperfections of the evolutionary theory, E. F. Tomlin, an enthusiastic amateur of natural philosophy, refers to its "fallacies". Tomlin does not think that evolutionary theory brought about great changes in human outlook, though he is inclined to agree with Stern that it inflicted a profound cultural shock. It was, however, so long in coming, and had been adumbrated so long before, that I myself regard it as a lesser cultural shock than the discovery of anthropoid apes in the voyages of discovery in the sixteenth century—in particular, the discovery of the anthropomorph *Homo Sylvestris Oran Utan* (Linn).

The burden of Tomlin's complaint is that "Evolution was an hypothesis which hardened into dogma before it had actually been thoroughly analysed . . . until Man's fortuitous and unaccountable advent [evolution] had appeared to lead nowhere, and this was not merely perplexing, but it placed man outside rather than inside the evolutionary process". I cannot criticise this argument because I do not understand it. Later, we are on more familiar ground: the inadequacy of

natural selection acting upon "fortuitous variations and mutations" to account for the accomplishments of evolution; but the reasoning that follows, which has to do with phylogenesis and ontogenesis, is so amateurish and inexpert that we are plunged into bewilderment again.

Tomlin's article does raise a real methodological conundrum: the genetical theory of natural selection is quite a difficult subject and full of traps, even for professionals. What can it be about evolutionary theory that gives laymen the confidence to make pronouncements about it of a kind they would certainly not make in the context of molecular biochemistry or geophysics? I imagine it is because having evolved themselves, they believe themselves entitled to express an opinion on the subject, much as they feel entitled to express an opinion on education on the grounds (often ill-founded) that they have been educated themselves.

I lost confidence in the threesome of Lacy, Weber and Pruitt writing on the "Edge of Evolution" when I read the opening paragraph, which draws a parallel between organic evolution and learning, and which contains the astonishing statement: "the learning process involves steps in which the person's brain passes from a completely naive state through a sequence of events until the person's brain has generated a mental construct of

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the details of some features of existence and arrived at a condition of comprehension." I do not accept this view, upon which their argument rests; I do not think that Chomsky would, and am quite certain that Immanuel Kant would not have done so either. The authors go on bravely to try to envisage molecular mechanism by which a cell might learn from its environment as we do when we learn to adapt to it. It is, of course, the very difficulty of this enterprise that has caused instructive theories of evolution to be supplanted by selective theories.

I do not believe that any scientific theory ever achieves the apodeictic certainty that puts it beyond the reach of criticism and if need be, of modification, and certainly I should make no such claim of the genetical theory of natural selection: surely some unpredictable innovation of principle has yet to be discovered before we can be completely at ease with current theories of the mechanism of evolution. In the meantime, following Dr Thomas H. Kuhn's view of the scientific process, the more thoroughly we investigate the prevailing paradigm—the prevailing constellation of received ideas and theoretical commitments—the more likely we are to come upon any empirical evidence that calls for a drastic revision of our beliefs.

Mourant's contribution ("Why Are There Blood Groups?") is a case in point. Mourant asks why, having regard to the fact that the only known function of blood group antigens is to cause diseases, blood group polymorphism is so widely prevalent, and is differently balanced in different populations? Mourant seeks an orthodox genetical interpretation in terms of the balance of selective forces, and points out that we are still woefully ignorant of the causes of the known association of blood groups with susceptibility to gastric cancers, to smallpox and to other infectious diseases. In two years' time, an article similar to Mourant's will probably be written about the relevance to disease of antigens that govern the outcome of tissue and organ transplantations, the more important of which, forming the major histocompatibility complex (MHC), occupy a well defined chromosomal segment.

Micklem is anxious lest modern immunology should degenerate into puzzle-solving, and proportionately anxious that the second Golden Age of immunology should bring practical rewards as great as those which followed from the first. In saying so, Micklem puts his finger on the greatest of all gaps in human understanding: how to translate thought into action when transforming our theoretical knowledge into practical results—how, in fact, a discovery may be premeditated and thus represent the fulfilment of a formulated intention. The advances Micklem looks for can only come with a still further deepening of our knowledge

of the immunological process, but luckily we do not have to know how the brain works in order to be able to use it. Appropriately enough, a distinguished subgroup of contributors to this volume have had no difficulty in making clear to us our lamentable ignorance of neurological learning and—"the great mystery"—of memory.

People with physical handicaps sometimes adapt themselves to them and lead reasonably normal lives. So it is with scientific understanding, and it is quite remarkable how prosperous biological science continues to be in spite of our deplorable ignorance of the mechanisms of embryonic differentiation and morphogenesis—the 'Old Trouble' indeed, and the subject of the contributions by Crick and Wigglesworth. This volume will achieve the highest hopes of its editors if the challenge embodied in these contribu-

tions brings about further recruitment into the field of developmental biology. The greatest advances in modern biology have grown up out of the intensive study of particular phenomena, such as pneumococcal transformation and protein synthesis in *Escherichia coli*; and I do not think developmental biology will prosper until the investigation of a morphogenetic process is equally intent. We do not know for certain why some cells stick together and others do not, but I do believe that mapping of cell surfaces in terms of the MHC is an important move in the right direction. And what, I wonder, is the relevance of the MHC to the phenomenon of contact inhibition? I am not at all sure that this problem has been studied in depth. □

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Cosmological Mafia

David Davies

The Encyclopaedia of Ignorance. Vol. 1: Physical Sciences. Edited by Ronald Duncan and Miranda Weston-Smith. Pp. 203. (Pergamon: Oxford, 1977.) Paperback £3.50. Hardback combined edition with Vol. 2 on the Life Sciences £10.

WHATEVER kind things one can say about the *Life Sciences* volume of the *Encyclopaedia of Ignorance*, the *Physical Sciences* volume must be rated a failure. The reason is simple—the book has fallen into the hands of the Mafia. Not, of course, the sort that operates rackets and encases its victims in concrete, but a brotherhood nonetheless—of cosmologists, almost entirely British, who between them have contributed more than half the book. Questions of curved space, gravitation, the origin of the Universe, the nature of time, and so on, are fascinating, but they occupy relatively little of the physical sciences spectrum; by taking up such a large proportion of space they leave precious little room even for particle physics, let alone solid-state physics, materials sciences, surface physics, low temperatures, oceans, atmospheres, the solid earth (a tiny contribution in the *Life Sciences* volume), optics, energy studies, chemistry . . . Most of these headings go completely unrepresented; a grotesque misrepresentation of what physical sciences are all about.

I suppose it could be argued that the book looks at the 'fundamental' problems of physical sciences (being about

the nature of things), whereas problems such as whether solar collectors can be made economical in the near future, whether earthquakes can be predicted, and how the climate will change, are of a lower order. It would be a singular snobbish point of view, and not one consistent with the *Life Sciences* volume, in which human nutrition and drug addiction find a place alongside evolution and molecular biology. More likely, no one sat down and started to worry about balance, while successive contributors passed on the names of their friends to the editors.

Nor is there much to be said for the quality of editing. In a preface (in which, amongst other things, the editors write "It could be said that science has to date advanced largely on the elbows and knees of technology. Even the concept of relativity depends on technology to prove its validity") the book is said to be aimed at the "informed layman, though possibly at university level". I would take this to mean that a history graduate should be able to read it, but do history graduates know so much about d'Alembertians, Hilbert space and degenerate gases that no word of explanation is necessary? Some of the contributors have clearly not been told at what level to write, and have written technical documents. Their contributions, rather than being skilfully edited, have simply been stapled together. One contribution, lifted straight from a research monograph on quantum gravity begins "A brief account of the Rutherford Laboratory Conference is followed by . . .".

Yet for all the failings of balance and editing, there are some nice things at an individual level. Amongst those who really are writing for the intelligent layman none sets a better example than Paul Davies on curved

space. There are some other thoughtful contributions, notably by Hermann Bondi on the folly of the pursuit of completeness, by Bill McCrea on the Solar System and by Alan Cottrell on emergent properties of complex systems. These and several others can

be read with profit by layman and scientist alike. What a pity that the book as a whole does not rise to the level of some of its parts. □

David Davies is Editor of Nature.

Glimpses of Rothschild

Edward Bullard

Meditations of a Broomstick. By Victor Rothschild. Pp. 187. (Collins: London, 1977.) £6.50.

THE romantic story of the Rothschilds has been told many times. The rise of Mayer Amschel and his five sons from the Frankfurt ghetto to European eminence as financiers, the founding of the English line by Nathan Meyer, their acceptance by Victorian society, the admission of Nathaniel to the House of Lords in 1887; these have been worked over many times by journalists and biographers. Now we have a book by Nathaniel's grandson Victor Rothschild, the third Baron, who is the inheritor of the family tradition and is one of the most entertaining and unpredictable men of his generation.

The first chapter is biographical; it gives an account of Rothschild's childhood and of his life at Cambridge up to the outbreak of World War II, and ends with a paragraph or two about his work with M15 during the War. The rest of the book contains reprints of speeches and letters to *The Times* covering the period since the War and concerned with a great variety of topics.

The first part about his childhood and schooling is fascinating—or is it just that it recalls memories to one also surviving from the same rapidly disappearing generation? What will today's readers make of the remoteness of parents, the big houses with "the army of nurses and governesses who tried to control us" or of the anarchy and brutality of the 'public' (that is, private) schools? That society now seems more remote than the Elizabethan world of disturbed teenagers knifing each other in tavern brawls.

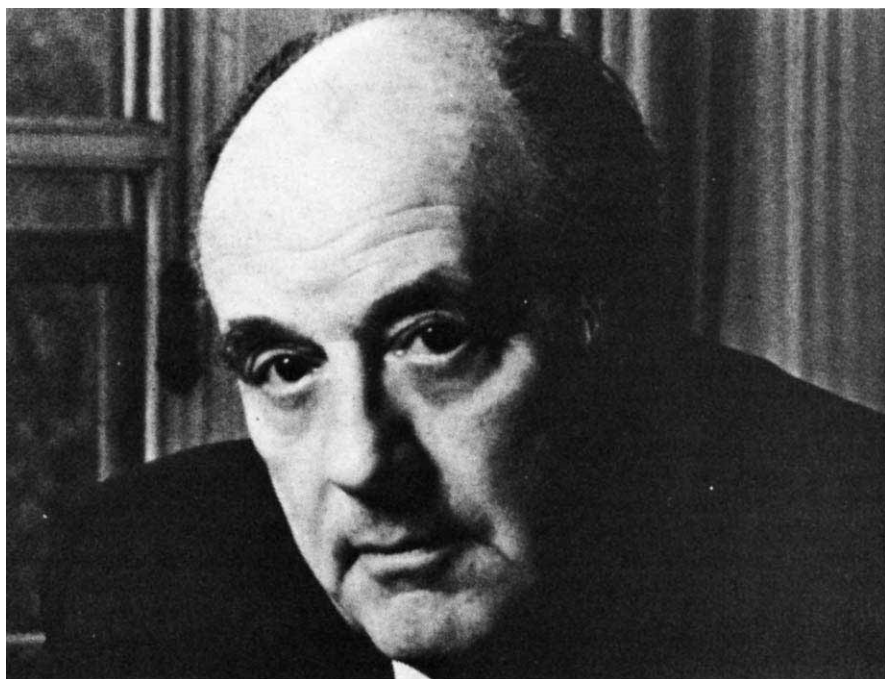
A curious feature of the description of Rothschild's Cambridge years is a list of Fellows of Trinity College, of whom he says he was scared. To be scared of Rutherford or, in a different way, of Hardy, is easily understandable—but was anybody ever afraid of Aston, Eddington or Coulson? A. E. Houseman might have been expected to make the list but does not.

One could wish that this first chapter were three times the length and that it were followed by a biographical account of Rothschild's adult life. As it is, the story must be pieced together from chapters with titles such as "Examination of a Crate of Onions near Northampton", "The Fertilisation of a Spermatozoan", "Too Old" and "Are You Fit to Make Decisions", and "The Infamous Rothschild Report".

The chapter about the onions is an account of the taking apart, in 1944, of a case of onions containing blocks of TNT and plastic explosive. It was dictated over a telephone line to a secretary at a safe distance. For this and similar exploits Rothschild was awarded the George Medal, though he does not mention it in the book. During the War many men undertook these tasks, and I suppose that most people had some idea how they would proceed if they became involved in such an undertaking. What is remarkable is not that he did these things, but that he did them without blowing himself up. Clearly he was at the same time bold, careful and sensible. His success contrasts favourably with the proceedings of a much more dashing member of the House of Lords who attempted to obtain explosive to feed a fire by hitting a bomb with a sledge hammer.

Most readers will probably turn first to the chapters on the "Infamous

Report". The report, entitled *The Organisation and Management of Government R&D*, appeared in November 1971 and has since been attacked with a violence which must have surprised even its author. Its main theme is that applied research should be aimed at meeting some specific need and that this is best ensured by a 'customer-contractor' relationship; that is, there must be someone who wants the work done and someone willing and able to do it. Rothschild points out that this statement follows logically from suitable definitions of 'applied', 'customer' and 'contractor'. The trouble arises in determining whether a given piece of work is applied, whether anyone does want it done and who are the members of the classes of 'customers' and 'contractors'. Rothschild answers that the customers are Government Departments, the contractors are the Research Councils or Government laboratories, and that a department demonstrates its desire to have the work done by being willing and able to pay for it. It is this part of the proposition that has caused the trouble. It suggests an almost adversary relationship between a technical group at a Research Council or Government laboratory and a primarily administrative department. All experience, particularly War-time experience, shows that things do not work this way and that you will only get effective and relevant R&D when there is a relationship of mutual confidence and a sharing of knowledge between 'customer' and 'contractor'. Obviously Rothschild knew this but, unfortunately, his report was too short to spell it out and seems to have been designed to irritate his scientific colleagues.



Victor Rothschild

The recommendations of the report are intended primarily to prevent large expenditures on projects for which there is no real need. It does not give the same attention to the equally important problem of ensuring that the things that are really needed are done. To suppose that all or most of such projects are among those for which some government department will spontaneously seek a contractor is unrealistic and contrary to experience. Striking recent examples abound in the field of energy R&D. Why was the geological disposal of nuclear waste not vigorously pursued in the early 1960s? Why were no serious efforts made to reduce the price of photo-electric cells from \$35 per watt to, say, one-tenth or one-hundredth of that amount, even after similar reductions had been achieved in the cost of transistors. Lord Rothschild should be encouraged to write a companion volume to his report covering these questions.

The book, like the report, is regrettably brief and sometimes not easy to understand. We learn, for example, that Lord Rosebery, a distant cousin, possessed "the best claret cellar in the United Kingdom including what was a famous tappit hen". Those familiar with eighteenth-century Scottish verse or, more accessibly with the Oxford English Dictionary, will know that a tappit hen can be either a chicken with a crest on its head or a jug. Which was it? The association with claret suggests the jug; on the other hand Harry Rosebery was fond of birds and, we are told, possessed a 'beloved parrot Inky'. If it was a jug why is it referred to in the past tense? Who dropped it? Such ambiguities have their interest; they lead the reader to consider many obscure questions. Why, for example, has the habit of calling for a jug of claret disappeared? Your reviewer has not encountered one since he was entertained by the RAF following the capture of the Luftwaffe's cellar near Brussels in 1944.

Rothschild says that he has been inhibited from writing about his time as overlord of the Prime Minister's 'Think Tank' by the restrictions placed on pronouncements by former civil servants. He may also have been inhibited by certain divergencies between his views on major questions and his position as a Labour peer. One illustration has survived in an article on the need for a farming policy; this is a slashing attack on the Trades Union Congress, or at any rate on its research unit.

A curious feature of the book is the author's understatement of his own achievements. His distinguished career in the physiology of reproduction ended when he was 48; of this he

says "I realised that though I could continue, with assistance, to do quite interesting experiments—my work was becoming monotonous and not too interesting. I therefore decided to stop".

The prime example of his denigration of himself is ingeniously concealed in the title of the book. Swift, as every schoolboy knows, wrote *A Meditation upon a Broomstick*. This was a practical joke played on Lady Berkeley, the wife of his patron. She required him to read to her passages from Boyle's *Occasional Reflections*, which Swift found tedious (they draw ingenious and pietistic morals from topics like *His Manner of Giving Meat to his Dog*). He therefore wrote a bogus meditation which Lady Berkeley accepted as a genuine work of Boyle. It compares a man to a broomstick, once "full of sap, full of leaves, and full of boughs: but now . . . handled by every dirty wench, condemned to

do her drudgery, and, by a capricious kind of fate, destined to make other things clean, and be nasty itself: at length, worn to the stumps in the service of the maids, it is either thrown out of doors, or condemned to the last use, of kindling a fire. When I beheld this I sighed, and said to myself "*Surely Man is a Broomstick*." Clearly this is to be taken as a joke; Rothschild does not really regard himself as Swift's broomstick.

Few men have had as varied a career as Victor Rothschild; a book that gives glimpses of his view of the extraordinary events of this century is welcome. Another book with more about how he sees it now and less of what he wrote to the editor of *The Times* or said in a Lords debate would be even more welcome. □

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Meteoric growth of American physics

Victor F. Weisskopf

The Physicists: The History of a Scientific Community in Modern America. By Daniel J. Kevles. Pp. 489. (Knopf: New York, 1978.) \$15.95.

THE reader from outside the United States should not be misled by the main title of this book. The subtitle is more accurate, as the book deals mainly with the American physics establishment from the 1870s to the present day, and its private and public support. Physicists outside America, their problems and achievements, are barely mentioned except in the unavoidable instances of quantum mechanics and relativity theory.

The development of American physics is an interesting topic. From a rather secondary effort, several steps behind the great European achievements before 1920 (with a few outstanding exceptions such as Willard Gibbs, Robert A. Millikan and Albert Michelson), it grew into the leading spearhead in practically all fields of physics later in this century. An anecdote told by I. I. Rabi (but unfortunately not reported in the book) characterises this meteoric rise. When Rabi came to Europe as a young post-graduate in the 1920s, there were important European institutions that asked to receive the *US Physical Review*

once a year in bulk shipment in order to save mailing expenses; later several copies of each new issue were sent by the fastest means to most physics institutes around the world.

The book attempts to tell the story of the rapid growth in American physics, but this worthwhile story is told in a somewhat one-sided way, by emphasising the managerial aspects and slighting the intellectual ones. Nevertheless, there is a lot of interesting information to be found in the book. Not many physicists in the US and abroad know about the tremendous efforts of Henry A. Rowland and some of his contemporaries in the 1880s to gain support for pure science and basic research for its own sake. They argued strongly for the formation of an elite devoted to "best-science". Rowland wanted "to create a science of physics in this country, rather than to call telegraphs, electric lights and such conveniences by the name of science . . .". His remark foreshadows a conflict between the great inventor Edison and those people who continued Rowland's quest for more basic and theoretical physics in the US. This conflict came to the fore in particular during World War I, when Robert A. Millikan and George E. Hale had to fight Edison, because he, in effect, ridiculed the scientific approach to practical problems. This most significant story is very well told.

The hard and uphill fight by Rowland, Hale, Millikan and many others really bore fruit only 50 years after it began in the 1880s. They had to resist not only the traditional leanings of the American public towards the practical, but also the inherent contradiction of the best-science elitism with the principles of democracy, a contradiction which complicated the development of science in the US from the

outset to the present. The author succeeds very well in describing these complications. He recounts how one cycle followed the other: the ups and downs of the best-science elitist attitudes emphasising basic research by the best people and institutions, alternating with claims for practical applications with immediate social pay-off and a more even spread of support to the institutions irrespective of excellence. The past decade, with its anti-science trends and its emphasis on problems of immediate relevance, is by no means a new phenomenon; neither was the great emphasis on basic science in the 1950s and 1960s. The author recounts similar periods earlier in history: for example, the period of anti-science in the late 1920s and early 1930s which have a strong bearing on what is happening today.

It is also interesting to learn of the importance of physics research in World War I, usually referred to as The Chemists War. Among the important contributions were marine detectors and flash-and-sound rangefinders for the pinpointing of enemy guns. That was the occasion when Millikan and Hale had to fight Edison in order to allow physicists the opportunity to work on these projects.

The glorious times of American physics started in the early 1930s. No longer did the ambitious young physicist find it necessary to work for a time in Europe. The account given of this change is factual and interesting, but the author does not attempt to get at the deeper reasons for this phenomenal change. He correctly emphasises that the influx of European refugee scientists and the political difficulties of European science have played only a contributory role. Probably, the primary cause was a change in the intellectual climate of the US; the spiritual soil became ready and fertile to support such a rapid growth of stature in the natural sciences. It may have had a lot to do with the spirit of the New Deal which brought with it a thorough democratic upheaval in higher education. A much larger number of gifted young people suddenly had access to knowledge and to the means of research. Some of them were attracted and fascinated by the new quantum physics. A few captivating and enterprising personalities established centres of learning and research in physics, such as E. O. Lawrence, I. I. Rabi, J. R. Oppenheimer, and refugee scientists such as H. A. Bethe and J. Franck. First-rate physics and physicists were produced at these centres at an amazing rate. The US took the lead in world physics, particularly in the fields of 'big physics' that needed team work and large resources.

The book then gives an account of the predominant role played by physicists during World War II, special emphasis being given to the development of radar

and the atomic bomb—stories often told before. We find here a compact description of the mobilisation of the scientists for War work, of the power struggles in Washington, of the establishment of the Office of Scientific Research and Development, of the eminent role of Vannevar Bush in all this, of the development of the Radiation Laboratory at MIT and of developments in nuclear physics, in particular at Los Alamos. The latter story is well told, but little is said about the contribution of those European scientists who came over to help.

The weakest parts of the book are the last chapters covering the 30 years after the War. These seem to have been written as an afterthought, in spite of the fact that this period witnessed a phenomenal expansion in physics, both in breadth and depth, all over the world. The account is restricted to happenings on the Washington scene; the political involvement of the nuclear scientists in the fight for national and international control of atomic energy; the H-bomb controversy; the Oppenheimer trial; the lavish support of basic science which, oddly enough, came primarily from the Office of Naval Research in the immediate post-War years; the effects of Sputnik on US scientific funding; and the slackening of support for basic science in the past decade. Among the achievements of physics, only those of 'big physics' (the developments in nuclear and high-energy physics) are reported, as they were closely related to the Washington scene. Achievements such as the laser, the theory of superconductivity, the fundamental advances in solid-state physics, statistical physics, and astrophysics, are not mentioned; nothing is said about the contributions of industrial laboratories such as Bell Telephone to the progress of physics so typical of the American scene; names such as Ph. Anderson, J. Bardeen, E. Purcell, J. Schwinger, Ch. Townes, R. R. Wilson, and others of equal importance, do not appear in the text.

There is practically no reference to what was going on in the world outside the US after World War II. That may be justified in view of the subtitle, which reduces the scope of the book to the American community. But one would have expected much more discussion of the remarkable impact of the expansion of American physics on physics abroad. In the first decades after the holocaust, many senior European and Asian physicists came to live in the US, attracted by relatively high salaries and excellent research opportunities. And this led to the well known brain-drain which was detrimental to science in the countries from which these scientists came. On the other side of the ledger, about 40% of the graduate students and postdoctoral scientists in the US before 1967 were foreigners paid from the lavish US support of science. Most of them did return, and the connections

and friendships which were established, helped a great deal in the revitalisation of science outside the US during the post-War period, particularly in Europe. The presence of so many foreign scientists in the US during their most productive and formative years was most instrumental in creating the cosmopolitan atmosphere and a spirit of international cooperation and discussion which characterised the post-War physics community. This was one of the most positive results of the generous financial support of American physics institutions. It also had some political consequences—for example, in the Pugwash movement for nuclear arms control. None of these interesting developments are mentioned.

Still, the book is well worth reading for what it does contain. The style is lively, clear and easy to read. The quoted facts are backed up by an excellent and extensive 'essay on sources' which not only lists all the pertinent literature but indicates their contents with useful comments. But the book that deals with the roots and causes of the phenomenal growth of physics during the past sixty years is not yet written. □

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Memoirs of a special assistant

Donald F. Hornig

Sputnik, Scientists and Eisenhower: A Memoir of the First Special Assistant to the President for Science and Technology. By James E. Killian, Jr. Pp. 315. (MIT Press: Cambridge, Massachusetts and London, 1977.) \$18.75; £10.50.

In 1957 the Cold War was at its height and the United States was very conscious of the role of science in determining the relative power of nations, particularly the power of the NATO countries *vis à vis* the Eastern bloc. Radar, sonar, the proximity fuze, the jet engine and nuclear weapons had already changed the nature of warfare. Long range rockets, the feasibility of which had been demonstrated by Germany during the war, were emerging as the nuclear weapons carriers against which there was no known defense. Furthermore, the confidence of the United States in its scientific and technological supremacy had been shaken when the USSR detonated a fission weapon in 1949 and a fusion weapon at substantially the same time as the United States.

Under these circumstances, the feat of the USSR in orbiting a 184-lb satellite, much bigger than that planned by the United States, on October 4, 1957, shocked the American public as well as the political and military communities. Its impact reverberated around the world as others re-assessed the technological and strategic balance and extrapolated what seemed to be extraordinary scientific progress by the Soviet Union to a fear of its impending military superiority.

Dr James R. Killian, Jr. was appointed the first Special Assistant to the President for Science and Technology in the aftermath of Sputnik. This memoir is an account of the events that led to his appointment by President Eisenhower, including his considerable previous contribution leading such efforts as the Technological Capabilities Panel, and his subsequent experiences in shaping a new place for science in the government of the United States. Although he kept no diary, the chronicle is meticulously documented and presents an intimate picture of the way the Eisenhower White House functioned and of the people who participated in it.

Dr Killian initiated the structure of a President's Science Advisory Committee, supported by numerous specialist panels, in the White House, together with science officers at the Assistant Secretary level in

the Cabinet departments, which was to continue until the White House science office was terminated by President Nixon in 1973. The longest section of the book is devoted to the work of the President's Science Advisory Committee (PSAC). It was a remarkable group of dedicated, talented people who worked very hard to analyse and understand numerous issues. It participated in and influenced significantly the debate which brought NASA into being and helped to devise a space program. It was deeply involved in the choices among missiles, including the decision to proceed with solid-propellant missiles such as Polaris. It played an important part in evaluating the scientific feasibility of a nuclear test ban. Among other matters in its far-reaching agenda were the formation of an Arms Control and Disarmament Agency, the evaluation of the nuclear-powered aircraft (the cancellation of which it did not recommend until several years later), the establishment of the Federal Council for Science and Technology to secure inter-agency coordination, and the conduct of a nuclear test outside the atmosphere. Throughout its life, it was deeply interested in the quality of higher education in science and encouraged federal support of basic research.

This memoir gives a picture of a smoothly operating government in which conclusions are reached and decisions taken with little rancour and none of the in-fighting which sometimes characterised the later White House with which this reviewer was acquainted. However, a somewhat different picture emerges from another work, *A Scientist at the White House* (Harvard University: Cambridge, Massachusetts and London; reviewed in *Nature* 266, 778; 1977), the daily diary of

Dr Killian's successor, Professor G. B. Kistiakowsky, who participated in many of the events chronicled in this memoir. In contrast to Dr Killian's detached, Olympian view, which recognises some disagreement and some error but rarely identifies stupidity and certainly not evil, Kistiakowsky's day by day account reveals a human struggle for influence and power in which ego and passion sometimes supplant generosity and reason. Dr Killian's memoir portrays an orderly world in which, for the most part, good sense prevails and the PSAC experiences little but success. Kistiakowsky describes a relative jungle in which advances are followed by retreats and progress is won painfully.

The two accounts supplement each other and together offer an excellent insight into the work of the science advisor and PSAC.

The advisory apparatus set up by Dr Killian is considered by many to be a model for the participation of science in government at the highest level. Dr Killian and the PSAC participated in difficult and important decisions and had the confidence of the President who referred to them as 'my scientists'. Yet, this influence was not maintained in later years; and as recently reconstituted, the office has been restructured and the PSAC eliminated. It is interesting to speculate on the reasons and Dr Killian's memoirs shed light on the factors involved. In part the times were opportune; in part the personal skill and integrity of Dr Killian gained him credibility and helped him to enlist devoted efforts from exceedingly able scientists. Furthermore, the PSAC agenda was focussed on defense, space and arms control. Correspondingly, the PSAC consisted largely of physical scientists with War-time and post-War experience with government and the military, men who knew each other and could work well together.

Now the science advisor must be concerned with economic growth, health, urban problems, and so on. He is asked to coordinate the numerous centres of scientific advice in the government and to provide oversight for a \$25x 10⁹ research and development budget. A homogeneous, deliberative PSAC for doing this is hard to imagine. Nevertheless, Dr Killian established the importance of scientific advice at the Presidential level and most of what he initiated has been perpetuated.

This clear and well written memoir ought to be read by anyone interested in science policy and the role of science in government. □



Eisenhower (left), Killian (right) and staff member (centre)

Donald F. Hornig served on the President's Science Advisory Committee from 1960-69 and was Special Assistant for Science and Technology to President Lyndon B. Johnson (1964-69). He is now Professor of Chemistry in Public Health, Harvard University.

Ancestral figure of computing

Anthony Hyman

The Mathematical Work of Charles Babbage. By J. M. Dubbey. Pp. 235. (Cambridge University Press: Cambridge, London and New York, 1977.) £12.50.

CHARLES BABBAGE is the great ancestral figure of computing. Where modern pioneers worked with colleagues and were part of a substantial movement, Babbage had to deal with all aspects of the problem himself: mathematics, technology, programming, applications, stating all the problems; even conceiving of the idea in the first place. And when he required finance (those who have difficulties with bodies offering grants might care to note) Babbage had to deal with the Prime Minister of the day.

Although he lived and worked in London and ample documentary evidence remains, most writing about Babbage's life and work has been muddled almost beyond belief. Thus, in discussing Babbage's life, without extensive study of widely distributed manuscript material, it is impossible to avoid errors, and this book contains its quota. Dr Dubbey says that Babbage was "almost certainly . . . a far different person from the one represented by popular misconception". One might go further: most of the pictures presented of Babbage have been little more than clumsy caricature.

The first years of Babbage's working life were devoted to pure mathematics. The history of this pioneering work, by Babbage and his friends Herschel and Peacock, which laid the foundations of the modern school of English mathematics, has hitherto been only sketchily known. Dr Dubbey's book, which is primarily concerned with Babbage's mathematical work before the commencement of the project to construct the first Difference Engine, does much to fill in the details of the picture.

At the beginning of 1812 Babbage took the initiative in founding the Analytical Society, which became the centre for his undergraduate work. Dr Dubbey refers to a letter from Bromhead to Babbage suggesting that there was a meeting of the Analytical Society as late as 20 December, 1817. However, I suspect this is merely Bromhead's graphic way of writing. He was feeling isolated in the country and was suggesting that it would be nice to revive the society: there was no-one at that 'meeting' but Bromhead himself. As an organisation the Analytical Society

functioned for a year and a half and produced only one publication, the *Memoirs*, whose cost was defrayed by the active members. Publication of the other books mentioned was solely the responsibility of the authors.

Dr Dubbey analyses Babbage's early mathematical work in considerable detail, making for his achievement bold claims which will long be discussed. He notes that before Babbage, ". . . problems arising from functional equations of higher order than the first had been almost overlooked. Babbage must be given full credit as the inventor of a distinct and important branch of mathematics". Dubbey claims that although the approach of Abel, Stokes, Weierstrass, Darboux and Hilbert to functional



Charles Babbage

equations ". . . is undoubtedly more rigorous than that of Babbage, it is doubtful if any of these could rival him in surpassing his contemporaries by the sheer ingenuity of method and generalisation of results". Dr Dubbey also implies that Babbage's papers on the theory of functions, of which he sent copies to Cauchy, stimulated Cauchy to solve the equations: $f(x+y) = f(x)+f(y)$; $f(x+y) = f(x)f(y)$; $f(xy) = f(x)+f(y)$; $f(xy) = f(x)f(y)$; which have been considered basic in subsequent theory.

The importance of Babbage's work on notation has long been recognised, but this book adds a good deal to what has been at all widely known.

Dr Dubbey shows convincingly that the basic ideas of the modern concept of algebra put forward by George Peacock in 1830 can be found in earlier work of Babbage's which Peacock had read in manuscript. In view of the habit which Babbage and his friends had of discussing such topics on every possible occasion when they met, there can be little doubt of the actual transmission of the ideas.

Of Babbage's rough draft *The Philosophy of Analysis*, abandoned when work on the calculating engines commenced, Dubbey concludes: "If he had developed the very fruitful ideas contained in the book . . . mathematical philosophy, modern algebra, the theory of games and stochastic mathematics [might well have been] developed many decades before they actually were". Babbage's influence on later English mathematicians, including De Morgan and Boole, and perhaps Continental mathematicians, may well have been greater than Dr Dubbey allows. I think he underestimates the importance of informal discussions at that time. Such channels of transmission are difficult for the historian to document but can be important.

This book treats other aspects of Babbage's work more sketchily. A discussion of the problems of Babbage's relations with government requires historical analysis, and that Dr Dubbey does not attempt.

His discussion of the Analytical Engine is based on the common misunderstanding that the Analytical Engine was a single machine. Actually, it was a class of machines, as is the Computer. A list of the classes of Analytical Engines was published a year and a half ago, perhaps too late for Dr Dubbey to take into account. He does not distinguish sufficiently clearly between a programmable calculator and a stored program computer. And his claim that "It has been described in chapter 8, how [the Analytical] engine was in every way comparable to a modern computer" really cannot be sustained. The Analytical Engine as described in this book is a calculator, albeit a powerful and versatile calculator, not a computer. A good case can indeed be made that Babbage did plan a computer, but the case rests on work of Babbage's which is not mentioned by Dr Dubbey.

There is a further difficulty in the illustrations. The Analytical Engine, the mill of which is shown here, is rather different from the Engine as it is described in this book. For example, it was probably not planned to use a punched-card system at all. Unhappily, the Analytical Engine is labelled "Difference Engine", and *vice versa*; and the transmission racks are shown sticking out wildly in positions they could never have reached in use.

The first seven chapters are the result of a great deal of painstaking work, well organised and clearly presented. Cambridge University Press is to be congratulated on publishing this study of Babbage's early mathematical work. One hopes that other detailed studies of crucial developments in the history of science will follow. □

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Scenarios of the past

Eric Ashby

Retrospective Technology Assessment, 1976. Edited by J. A. Tarr. Pp. 326. (San Francisco Press: San Francisco, (547 Howard St), 1977.) \$15.50.

"PEERING into the future is a popular and agreeable pastime which, so long as it is not taken seriously, is comparatively innocuous". This was written only 20 years ago, by J. Jewkes, D. Sawers and R. Stillerman in their authoritative book *The Sources of Invention* (Macmillan: London, 1958). For better or for worse, peering into the future is now being taken very seriously indeed. In the USA, the National Environmental Policy Act of 1969 obliges planners of many projects to forecast the effects on the environment of their plans; within two years of passing the Act, no fewer than 3,635 environmental impact statements had been lodged with the Council on Environmental Quality. And the Office of Technology Assessment, set up in 1972, has the job of advising Congress about the second order effects of innovations in technology. It has come in for some criticism (summarised in *Nature* recently: 268, 4, 1977) but the need for technology assessment is not disputed; the case in favour of some organisation to undertake assessments was convincingly made by a panel of the National Academy of Sciences, in a much quoted report published in 1969: *Technology: Process of Assessment and Choice* (US Government Printing Office: Washington, DC, 1969).

In Europe, the initiative for taking technology assessment seriously came from OECD (E. Jantsch, *Technological Forecasting in Perspective*, OECD: Paris, 1967), in 1969. The most recent manifestation of interest in it is to be found in the report *Europe Plus Thirty* (Brussels, 1976) commissioned by the Council of Ministers of the European Community, which includes among its recommendations a "long-term forecasting instrument" to carry out forecasting and technology assessment. On the environmental front, there is already formal provision for environmental impact assessments in Germany and Ireland. The Department of the Environment has published a thorough examination of the ways in which environmental impact assessments might be introduced in Britain (Research Report, II: *Environmental*

Impact Analysis, HMSO: London, 1976), and the Commission of the European Communities has given notice that it will be proposing Community procedures for assessing environmental impact (*Environmental Programme*, 1977-81; Bulletin, suppl. 6/76, Brussels, 1976), these, if they are approved, will become mandatory for member countries of the Community. Far from being an 'agreeable pastime', peering into the future is becoming a profession as respectable in the twentieth century as soothsaying was in ancient Rome.

Technology assessment is performed not out of idle curiosity but in order to influence political decisions. Therefore, the first question which has to be asked about it is: Is it reliable? It has a dismal track record. No-one, at the turn of the century, forecast that if the use of automobiles increased exponentially they would by the 1970s kill or injure some 340,000 people a year in Britain alone; and if that forecast had been made, would that have influenced government policy on road transport? No-one, in the early nineteenth century, forecast that the water closet, which was welcomed as a device to reduce the risk of disease in the home, would in fact spread disease by polluting the rivers from which people drank. Even when sophisticated technology assessments are made deliberately and at considerable cost (as they were for the Volta dam in Ghana), the assessments fail to forecast quite serious social consequences. In the present state of the art, technology assessment is not reliable enough to be of much use in policy-making. The most urgent need, therefore, is to assess technology assessment itself.

These reflections arise from a study of *Retrospective Technology Assessment*, 1976 which records the proceedings of a conference held in December 1976 under the sponsorship of the National Science Foundation and the Carnegie-Mellon University. The purpose of the conference was to study the role of history in technology assessment. The 18 papers and a summary of the discussion cover a diversity of topics, including methodology, eight case-studies, and four essays on technology and values. The message of the papers on methodology is that there is no methodology: the best course is to use any data you can put your hands on: to accept subjective hunch as readily as you accept statistical forecasts.

The case-studies are the most significant contributions; significant for two reasons: first, because there are so few of them to put on record, and second, because the few case-studies which have been seriously made do show that they

might, if pursued, illuminate some present-day problems in technology assessment. They fully justify the decision of the National Science Foundation, made in 1975, to finance four major studies in retrospective technology assessment: on submarine telegraphy, the treatment of waste water, the telephone, and the development of corporate management (a social technology).

These studies are not yet complete, but parts of some of them are included in this book. If they turn out to be useful for policy makers, it will not (of course) be because history repeats itself (which it doesn't), but because there may be common principles underlying the process of diffusion of a new technology into society. It is too early to speculate about these principles (if indeed they exist), but the case-studies do throw flickers of light here and there. Thus, it was predictable that when the Erie Canal was built between 1817 and 1825, settlements would grow up along the canal. What was less predictable was that the local roads would deteriorate, that this would make difficulties for farmers in Upper New York, and that agriculture in the area would suffer because wheat could now be brought cheaply through the canal from the richer farmlands in Ohio. In another study, on the consequences of bringing piped water to homes in American towns, it was clear that no-one gave sufficient thought to the problem of how to get the water out of the homes into which it had been piped. Sewers were for carrying storm water out of the streets, and it was for many years illegal to put the wastes from water closets into sewers. When this ban was finally lifted, one consequence was that something which had from time immemorial been the responsibility of the householder became the responsibility of local government. There was great inertia to be overcome before this shift of responsibility could be achieved; bond issues to pay for the building of sewage systems were resisted by some people on the ground that they were committing future generations to a new fangled way of dealing with human wastes, and by other people on the ground that the traditional way of dealing with human wastes—to use them to fertilise crops—was the 'right' way and should not be changed.

The theme of inertia runs through several of the case-studies. The most impressive example of it is in the essay on the impact of the Atlantic cable on diplomacy. In 1866, when the cable was fully operating, only five messages were cabled between the US Department of State and the US Legation in London. Up to the turn of the cen-

tury, the volume of correspondence between the two posts rarely reached 150 messages a year, compared with 500–1,000 written despatches. It was only after 1938 that the total counts of cabled messages persistently exceeded the total counts of letter correspondence. Opinions about the values of the cable system to politics differed; some believed that cables spread rumours, others believed that cables were valuable to de-fuse dangerous political incidents. What is clear is that the commercial world adapted itself far more quickly to the Atlantic cable than the diplomatic world did. There were similar symptoms of inertia in the development of a national policy for electric power in the USA (even the electrical engineers could not bring themselves to make an unambiguous recommendation as to whether direct or alternating current should be used); and in the planning (or, rather, lack of planning) in the siting and construction of airports (this was an example of an almost message-proof curtain between the designers of aircraft and the planners of airports).

The last two essays in the book give some hint as to the deeper difficulties in the applications of technology assessment, even if the assessments could be relied upon. One has to ask not only

what effects will supersonic travel, video-telephones, oral contraceptives—all these material technologies—have upon patterns of society; one has to ask also what social institutions can be created to handle the consequences of these material technologies. For it is to the social institutions of a nation that one looks for guidance about ends. Technology assessment should not be allowed to become (to use Robert Merton's phrase, quoted in a perceptive essay by Rondo Cameron in the book) "the quest for continually improved means to carelessly examined ends".

Retrospective Technology Assessment has one important lesson for people who aspire to write scenarios for the future. It is that they would do well to begin by closely studying some episode in the past where technology has changed patterns of life. This may not give them formulae for forecasting future events but it would surely persuade them to present their scenarios to the world with more humility and less confidence than some of them exhibit at present. □

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Soviet technology

Christopher Freeman

The Technological Level of Soviet Industry. Edited by R. Amann, J. M. Cooper and R. W. Davies. Pp. 575. (Yale University Press: New Haven, Connecticut and London, 1977.) \$30; £20.

THIS volume is a landmark in the study of Soviet technology. There have, of course, been many previous studies, but this is a far better book than any of its predecessors, both in terms of the information which it conveys and the quality of the analysis.

In the Western literature, probably Sutton deserves the credit for the greatest originality in his study of Soviet imports of foreign technology, but his work was marred by the depth of his unscholarly political bias against the Soviet system. Even less helpful were some of the official apologetics for the regime.

The Birmingham study is remarkable not just for its massive scholarly achievement, but even more for its success in linking together the contribution of technologists, economists, scientists and political scientists. The methodological

problems were and remain very great. Not only are there the usual problems of barriers arising from differences in language and culture, but these are compounded by the difficulties of measuring technical change or technological achievement in any language or culture, without such cooperation.

The Birmingham team avoided the temptation to escape from these difficulties by two well-known pseudo-academic techniques: the retreat into a fog of mathematical complexity in one specialised discipline without resolving the real problems, and the parade of massive technological detail to disguise inability to make any generalisation. The important thing about the Birmingham study is that the wood and the trees are both there. Nine chapters discuss in depth the evidence of specific industries (steel, machine tools, electric power transmission, chemicals, computers, tanks, missiles, rockets and process control equipment). Each of these chapters ends with a succinct conclusion summing up the achievements of Soviet technology by comparison with other leading industrialised countries in the particular sector considered. The first two chapters (by Amann and Davies) discuss the difficulties of generalisation but nevertheless come to quite clear conclusions on the basis of the entire evidence assembled and other (more aggregative) data.

It is not possible in a short review to do justice to any of the specific sector studies. Many different authors contributed, and obviously there is some unevenness in the quality of the available data and the use which is made of it. Nevertheless, they all show evidence of a determined attempt to come to grips with a great variety of original Soviet source material and of a consistent team and editorial effort at interpretation and synthesis. In all cases an effort was made to obtain and discuss data on the level of research and development, the dates of first introduction of key advances, the rate of diffusion of key innovations, technical indices, foreign trade and technology imports. On the second and third of these measures, the efforts were on the whole successful, but much less so in relation to the other indicators.

The main conclusion, in Davies' own words, is that: "in most of the technologies we have studied there is no evidence of a substantial diminution of the technological gap between the USSR and the West in the past 15–20 years". It is important to emphasise that this conclusion is not based on unreliable macro-economic aggregate production function analysis, with all the pitfalls which the authors so wisely avoided; nor yet on comparative specifications of individual machines, instruments or plants with the impossibility of satisfactory generalisation. It is based on carefully assembled data on the date of introduction of what are internationally accepted key technical innovations and the rate at which these spread through the sector in question by comparison with other major industrial countries. The authors themselves are at pains to emphasise the qualifications and reservations applying to their own method, and whenever possible they apply other checks and additional indicators, but it is so superior to the alternative methods that it puts this study in a class by itself.

Neither the editors nor any of the other authors make any attempt to go beyond their brief and assess the broader significance of their main findings. A reviewer may be permitted the luxury of going further and speculating about three of the implications, in ascending order of importance.

First, this approach and these results were possible only because the Birmingham research was organised in a certain way. The book itself took more than five years to produce, and in one important sense it was the outcome of 15 years' specialisation on the Soviet research and development system, and Soviet technoeconomic policies by the Birmingham Centre for Russian and East European Studies. Their earlier major publication on Soviet science policy was also a landmark in its time, but this book represents the mature achievement of a prolonged process. However, their specialisation

was not a disciplinary one but a problem-based specialisation. The Social Science Research Council are to be congratulated on their 4-year grant in support of this project, but they must surely give further thought to the whole question of interdisciplinary team research, and how it can be sustained in British universities. When are we going to see a similar study of technology in British industry?

Secondly, the findings of the Birmingham research lend strong support to the conclusions of recent American work on the level of Soviet military expenditures which have been relatively stable over a long period. There has been no sudden great new "threat" from spectacular technological advances in the USSR, either in the civil or the military sphere. Japan and West Germany have made far more spectacular progress in closing the "technology gap" with the US than the USSR. Certainly, the Soviet Union remains a military super-power and does concentrate immense resources on military innovation and production. Its achievements are greater in this field than elsewhere, and there

is every justification for the view that progress towards disarmament will be secure only when it encompasses military research and development as well as existing weapon systems. Nevertheless, the overall performance of the Soviet economy and Soviet technology are not such as to justify some sudden massive increase in NATO military expenditures, as some alarmists have been suggesting.

Finally, the experience of Soviet technology decisively refutes C. P. Snow's cynical view of the lack of any association between democracy and the progress of science and technology. The association is a complex one and a long-term one, but the Birmingham study lends overwhelming support for the view of those communists who have been arguing that the principal constraint on the progress of the Socialist economies is the lack of initiative, participation, and independent criticism at all levels of economic management, politics and science. □

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Nuclear power jubilee

Duncan Burn

Nuclear Power: Its Development in the United Kingdom. By R. F. Pocock. Pp. 272. (Unwin Brothers: Old Woking, UK, 1977.) Hardback £12.80; \$25; paperback £5.10; \$10.

It is 25 years since the construction of the Calder Hall plant was started, and this book, sponsored by the Institution of Nuclear Engineers, is a jubilee history of the nuclear power industry. The author is a nuclear engineer and the book is concerned largely with questions of organisation, economics and politics. The Queen, opening Calder Hall in 1956, said: "It may well prove to be among the greatest of our contributions to human welfare that we led the way to demonstrating the peaceful uses of this new form of power". This is quoted on the dust cover, but in the book the theme that Britain led the world plays a small part. Dr Lewin, President of the Institution, says in his preface more circumspectly that the story is "a record of achievements of men who can take pride in it", but "also of things that went wrong".

It is impossible to conceal that things went wrong—the crucial questions are when, how and why? I find Mr Pocock's account unsatisfactory on these questions. He is reticent; Dr Lewin says he "avoids pillorying individuals, some not only alive but still working in the industry. He prefers to let the facts draw the lessons for

themselves". Facts never draw lessons, lessons must be drawn from them. When decisions are examined in retrospect the quality of those who made them, the institutions which gave them power, their advisers, their knowledge of alternatives, the competition, are relevant facts. But reticence is not a major source of my worries. The basic trouble is that Mr Pocock seems to think the main things which went wrong were right in their beginnings. He pays too much respect perhaps to the founding fathers in the UK Atomic Energy Authority (UKAEA) and government. He joined the nuclear industry in 1966, so was not in it when the crucial early judgments were made.

His treatment of the decision to launch in 1955 a 10-year programme of nuclear plants with a total capacity of 1500–1800 MW, raised in 1957 to 5000–6000 MW, all from Magnox plants is indicative. This was so large that "only a comparatively small proportion of the total nuclear effort could be devoted to possible alternative reactors." The 1955 decision Mr Pocock states was "not a long term solution to possible future problems; it was the sole existing answer to immediate needs", and asserts that "the economic cost of providing the nuclear capacity was not really a factor in the Ministry's decision in 1955". Some experts thought Magnox reactors might fairly quickly produce power as cheaply as fossil fuel plants, but he thinks their impact was slight. My experience at the time gave me a different picture.

In any case the view that the 1955 programme "was the sole existing answer to immediate needs", which had some influence for a short time, was a fantasy and soon seen to be so. Mr Pocock seems to

treat it as though it were right, and vindicated by the arrival of the energy crisis in 1973. The only existing answer to immediate needs in 1955 was oil. It proved a good answer. But increasing dependence on Middle East oil involved a political risk, and the supply would not expand indefinitely; these were good reasons for developing nuclear power, but not for plunging into a large programme without regard to costs.

America and the Continent were at risk in the same way. The Paley Report of 1952 set 1975 as the date when the US, already importing much crude oil by 1952, would need a supplementary energy source, nuclear energy being the most promising. The US Atomic Energy Commission did not, as Pocock suggests, promote competing reactor systems because it could take its time; it believed this the right way to get low cost systems quickly. Experience justified it. For a time, the author says, Britain had far more nuclear capacity at the cost of relying on an obsolescent system. "When the early experiments had been completed the US was able to erode that lead and establish a commanding technical and commercial position". He calls this "the logical outcome of the situation in 1954", but Britain's performance was an outcome of faulty judgments, a premature choice of system, a premature programme: there was nothing inevitable about it. Most Continental countries followed the American pattern, and went ahead. A later choice of system could have provided a stronger nuclear industry to face the crisis of 1973.

All this has great significance for the discussion of Ministerial participation, UKAEA monopoly and power seeking, and centralisation in general, on which Mr Pocock writes interestingly but not in this context. He is understandably disturbed at vacillations in energy policy and seems to regard the slowdown in ordering obsolescent Magnox plants from 1960 as inconsistent with the premises of the 1955 policy, which he over simplifies. But since the cost and market forecasts had proved wrong it would have been irresponsible to act otherwise. He quotes figures published in 1971 which it was claimed showed that Magnox generating costs were lower than the cost of power from the best coal-fired plants, as though these proved that forecasts of low costs had been right, but fails to point out that the 1971 figures were "accounting costs" only, with no adjustment of capital costs to reflect inflation. The Department of Trade and Industry pointed out that after this adjustment Magnox costs were no longer the lowest. This is one of several points where the criteria used in his economic judgments are inadequate. He refers to the contribution by nuclear plants of about one-tenth of the electricity by 1975–76 as marking a quiet revolution but does not add that this was much less than

had been widely expected; with a different development policy this would presumably have been raised. He says of the UKAEA's 1957-58 plan drawn up by a group with a former Treasury planner as chairman, which concentrated development on the advanced gas-cooled reactor with the steam-generating heavy water reactor as a reserve, and ended work on the pressurized water reactor, that it was "well planned to make the best use of available resources, provided that technical economic and political conditions did not change". How does he decide it was well planned? Was the proviso realistic? Or is it an implicit criticism? The resources absorbed in the programme in 20 years with no return at all until 1976 have been enormous. He says much on what went wrong, but gives no comprehensive measure of the vast cost of the

programme in current prices, such as Professor David Henderson has published.

I have concentrated on illustrating features of the book which in my view limit its value in analysing the problems of organisation and Ministerial intervention and assessing the gains so far from nuclear programmes. I would add in conclusion that I have found it attractive, stimulating rather than provocative, and admirably broad in scope. It deals well with many topics outside the programmes—the Windscale incident, nuclear ship propulsion, growth of safety organisation, 'waste' and the environment, for example; and its account of the consortia is particularly good and, with good judgment, more appreciative than is usual. □

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Energy policy

Walt Patterson

Energy Policy: Strategies for Uncertainty. By P. Lesley Cook and A. J. Surrey. Pp. 240. (Martin Robertson: London, 1977.) £8.50.

CONTEMPORARY daredevils have lately come up with two new and hair-raising ways to tempt fate. One is skateboarding. The other is energy policy. Both activities can induce in their participants a brisk and delicious exhilaration. Both, however, bring with them the possibility of coming an abrupt and messy cropper. It is undoubtedly desirable to don protective apparel, but no amount of protection can guarantee immunity from disaster.

Lesley Cook and John Surrey have no advice for skateboarders. But they have written a brilliant and vivid depiction of the pitfalls gaping before those intrepid souls who would venture on to the sweeping contours of energy policy. They begin by delineating the background which has given rise to the preoccupation in the 1970s with what is now called 'energy policy'. Here as throughout the book they focus primarily on the UK; but even that constraint in no way leaves them short of monitory historical examples to set the stage. They date the prehistory of 'energy policy' to the White Paper of 1967 on Fuel Policy, which might have been written by Etruscans, so remote are its assumptions from those now coming to the fore.

The subtitle of the book is *Strategies for Uncertainty*. Chapter 3 identifies and disaggregates many general categories of uncertainty which now confront planners. Part II (chapters 4-9) dissects in detail the overall present-day UK position, and the particulars affecting oil, coal, gas, and electricity, and their interdependence. After the first 149 pages no reader can

have any doubt about the quantity and variety of uncertainty prevailing.

About problems of forecasting, planning of investment, establishment of performance criteria, and coordination of policies between supply industries, Cook and Surrey are incisive and unsparing. It is a pleasure to report that, although both authors are economists, they write crisp and readable prose, commendably free of jargon and accessible to any reader with a modicum of interest in the subject. Indeed any reader who comes to the book without previous involvement may well find the book a stimulus to further interest, because the authors obviously care about the issues they are discussing. Their generalisations are almost invariably drawn from specific—often recent—examples, giving an immediacy to what might in other hands have been a bewildering academic exercise.

Cook and Surrey pinpoint so many full-fledged problems that it is almost sadistic to ask them to note still more. Unfortunately, however, the 'uncertainty' of Part II is a great deal more convincing than the 'strategies' they propose in Part III. Their analysis in Part II, although by far the most comprehensive yet presented, nevertheless falls short. In doing so it undermines Part III. The difficulty arises because Cook and Surrey, despite their perspicacity, approach the energy question almost exclusively from the viewpoint of those responsible for the supply and distribution of fuel. What they are discussing is accordingly not 'energy policy' but fuel policy. That is, to be sure, a necessary and important area for study. But it is not coextensive with 'energy policy', as energy policy is now coming to be understood. The drawback is especially evident as it affects the commendable proposal by Cook and Surrey that future planning should start with a long-term strategy, and then adopt medium- and short-term strategies in keeping with the long-term. It is not, however, possible to

develop a long-term strategy without taking far more account of the ways in which fuel energy is used, and of the policy measures which affect its use.

Recent work in the UK and elsewhere has begun to build a data base about the use of energy—domestic, commercial, industrial, transport; about the temperature spectrum of energy requirements; about the time variations of uses; about the technical improvements by which the same end-uses can be accomplished with far more elegance and economy of means; and about the institutional and policy measures which may be implemented to bring about an entirely new approach to the role of fuel energy in society. To mention but one example, the Energy Project of the International Institute for Environment and Development has prepared detailed, disaggregated and costed scenarios which suggest that UK primary fuel energy use in 2025 need be no more than half that in 1978, while standards of personal comfort, mobility and industrial productivity are higher than those of today. This and other similar analyses are now being given serious consideration by the Energy Technology Support Unit of the UK Government Department of Energy. Until such possibilities are incorporated in 'energy policy', alongside considerations affecting investment and planning for additional supply, any 'energy policy' can only be partial. From the point of view of social objectives and values, such a partial policy will be inevitably far from 'optimal', and almost certainly unnecessarily vulnerable; this would include the policy approach proposed here by Cook and Surrey.

In fairness, however, it should be noted that one of the most active and imaginative contributors to the new ferment of innovative energy policy thinking is the Science Policy Research Unit (SPRU) at the University of Sussex, of which John Surrey is a Senior Fellow. SPRU's work on energy use, and disaggregated forecasting, has been a major factor in the gradual enlightenment now dawning at the Department of Energy, and demonstrated—if as yet somewhat tentatively—in the recent Green Paper on Energy Policy.

Cook and Surrey completed most of the writing of their book before the end of 1976. They can hardly be faulted if it has by now been slightly overtaken by the unravelling 'uncertainty' which they have foretold.

Indeed, if 'energy policy' is—as seems likely—soon to embrace building standards, transport systems, industrial production and processes, agriculture and land-use planning, and a host of other policy areas, it may before long have outlived its fascination as an identifiable activity. Anyone for skateboarding? □

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Popularising black holes

Roman Znajek

The Collapsing Universe: The Story of Black Holes. By Isaac Asimov. Pp. 204. (Walker: New York; Hutchinson: London and Sydney, 1977.) £4.50.

POPULAR books on black holes seem to consist of either metaphysics or science fiction. It is pleasant to discover that Isaac Asimov's *The Collapsing Universe* is mainly about astronomy. It is perhaps a bit surprising as well, because Asimov is of course a self-confessed "science-fiction writer of some repute". His book is an attempt to convey non-trivial information about black holes to a readership whose previous knowledge does not extend beyond an ability to multiply. Writing as someone whose social life is occasionally blighted by requests to explain what it is I do, I can only state that such a book is highly desirable.

Before a layman can appreciate what black holes are and how they fit into the general scheme of things, he must first learn some basic facts concerning the objects to be found in the Universe and the forces that act between them. This takes time, and it is therefore quite proper that most of Asimov's book is not about black holes at all. In chapter 1 Asimov describes the four forces that govern the Universe. Of these four, only gravity and electromagnetism are important over distances larger than the diameter of an atomic nucleus. Asimov carefully explains the inverse-square law of force in a manner that should be comprehensible to a pupil considered incapable of taking ordinary level school physics. An advanced level school student would be in danger of getting seriously bored. Electromagnetism is intrinsically stronger than gravity, and it is electromagnetism that controls virtually all terrestrial phenomena. (Gravity only holds things down.) But gravity does not have the self-cancelling effect inherent in electromagnetism; gravity finds it easier to act over large distances, and it is therefore gravity that determines the motions of planetary systems and galaxies.

Chapter 2 is about the planets. Their masses, escape velocities, densities and other properties are described in considerable (though elementary) detail. Chapter 3, on compressed matter, explains why the planets do not simply collapse under their own weight. Electromagnetic forces between adjacent atoms are strong enough to overcome the total gravitational force acting between all the atoms in the planet. The two forces are at an "eternal standoff". This is not the

case with stars; but stars are hot, and it is their heat which results in an outward pressure that balances gravity. There is indeed a standoff, but it is only temporary. Stars radiate their heat away, and must burn nuclear fuel to keep going. The fuel supply is finite, and sooner or later the stars will cool and contract. Even then, total gravitational collapse can be halted by the degeneracy pressure of electrons and neutrons, which does not depend on heat. The resulting objects are white dwarves and neutron stars, and these are described in chapters 4 and 6 respectively. The intermediate chapter links the big bang, stellar evolution and supernovae under the conveniently general heading of "Exploding Matter".

So far, so good. The reader has been given an elementary introduction to a great deal of astronomy. Asimov is clear and usually accurate. (He does seem to be somewhat confused about the relationship between ionisation and degeneracy.) Rather than just describe what astronomers have concluded, he outlines the way in which they have arrived at those conclusions.

This is after all a book on black holes, and they are the subject of the final two chapters. Asimov's treatment of black holes is disappointing. He states that their escape velocity is equal to the speed of light and that "it so happens that physicists are quite certain that no physical object possessing mass can move at a speed equal to or greater than light". This is uncharacteristically dogmatic. A spacetime diagram displaying the motion of light rays in and around a black hole would explain what he means by the escape velocity being the speed of light, and would not demand any more of his readers. Even better, a chapter containing a qualitative description of special relativity would explain why physicists can be so certain that nothing moves faster than light, and would be very worthwhile for its own sake. There are in fact no illustrations in Asimov's book, and these would surely be a great help.

The basic trouble seems to be that Asimov is stronger on observations than on theory. Black holes are still a rather theoretical subject. His account of the discovery of the X-ray source Cygnus X-1 is fine, and so is his description of the reasoning that leads many astronomers to believe that Cygnus X-1 contains a black hole. What Asimov does not do well is to explain the accretion disk mechanism that is supposed to be responsible for generating the X rays. He says that particles spiralling into the hole lose gravitational energy, but nowhere does he try to explain what gravitational energy is. One or two down-to-earth examples, such as a stone falling into a well, would help. The vital role of differential rotation and friction in generating

the heat that produces the X rays is not mentioned.

Asimov presents a thoroughly oversimplified version of the Penrose process. With an additional half-page of text he could have got it right. He seems to think that the Penrose process is controversial. Indeed, he goes so far as to state that "almost anything some astronomers suggest about a black hole is denied by other astronomers". This is probably correct in so far as he refers to theories concerning the presence of black holes in specific objects such as quasars or radio galaxies. The general properties of black holes are, however, the province of mathematics rather than physics. Given the fundamental axiom that spacetime obeys the laws of general relativity (and if you are going to write a book about black holes you should at least have some faith in the fundamental axiom) then the properties of black holes are described by mathematical theorems. The Penrose process is such a theorem. Another theorem (which requires one to accept quantum field theory as well as general relativity) is the Hawking effect whereby small black holes emit thermal radiation and evaporate. This generally accepted result is probably the most interesting and significant piece of work carried out on black holes in the past few years. According to Asimov, it is a speculative suggestion, and he only gives it two or three lines. The unimportant and probably incorrect idea that the great Siberian explosion of 1908 was due to a mini-black hole passing through the Earth is given a whole page.

The usual trouble with popularisations is that they treat speculation as established knowledge. *The Collapsing Universe* is quite exceptional in having the opposite tendency. Asimov is clearly acting from the best of motives. He cannot be accused of sensationalism, although whoever wrote the blurb on the dust-jacket can. Asimov is aware of many of the developments that have taken place in the past few years, but he seems to have little idea of their relative importance. This could be due to haphazard communication between writers like him and the mainstream scientific community, at least as far as black holes are concerned. One possible remedy might be to hold meetings between representative groups of scientists and the writers and journalists whose job it is to present science to the general public.

To sum up, Asimov has written half of a good book on black holes. He ought to have the opportunity of writing the other half, and I look forward to seeing a second edition. □

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Rundown Universe?

Michael Rowan-Robinson

The Runaway Universe. By Paul Davies. Pp 205. (Dent: London, 1978.) £5.95.

The Runaway Universe is an ambitious book which describes the evolution of the Universe from the quantum era 10^{-43} seconds after the big bang through to the future of a billion billion years hence. Written in non-technical language, it describes the emergence of order out of chaos and the inevitable ultimate descent to disorder.

Popular science books serve an important purpose not only for the public but also for science itself. Most of us who work in science have been profoundly influenced by the popular science books we read in our youth. The books now being written, and the television and radio programmes being prepared, will help to condition the kind of scientists and the kind of science we have in a decade's time.

Yet it is not easy to say what makes a good popular science book. How are we to compare a book written in his spare time by a professional scientist, for example, with one by a creative writer? The scientist will know from the inside one particular area of science, but may be struggling when it comes to getting this across to the general reader. The writer may have a shallow understanding of any particular science, but can overcome this with a broader perspective on science as a whole and a far greater articulateness. In between there is the science journalist, who usually has some background in science itself and often has a better idea of what is going on in science as a whole than the average working scientist. He may therefore combine the best of both worlds of science and writing (or he may fall between two stools).

The scientist who wants to write or talk about his field and the general reader, are both at the mercy of the publisher and media mogul. The latter usually have a fixed idea of what a science book or programme should be like. They also cannot be immune to the whims, fads and superstitions of the public. In the past decade there has been a spate of books exploiting this superstition in the most shameless way, and not all by the Velikovskys and von Danikens of this world.

The most controversial aspect of Paul Davies' book is the credence he

gives to the possibility that the Earth may have been visited in the past by extraterrestrial beings. As Paul Davies is a prominent young relativist and cosmologist who has made a significant contribution to ideas about quantum gravity and has written a most impressive and valuable research level book *The Physics of Time Asymmetry*, his treatment of UFOs is rather different from that of the many offerings on the book stalls. He treats the claims of UFO sightings and landings with some scepticism, pointing out that "it is scarcely conceivable that another community should engage in a massive expedition across the galaxy only to land in a potato field in Wiltshire for a few minutes and then return across several hundred light years of space". At the same time he also gives a lot of encouragement to the believers: "No systematic evidence has ever been produced to show that the eyewitnesses were liars or subject to delusions. The reports are made by a fairly typical cross-section of the population, but include astronauts in orbit, pilots and astronomers", and so on. He also has a nice line in unmanned biological probes, which arrive on a planet as seeds, sprout eyes, ears and radio transmitters, and send the glad tidings back before being eaten. Paul Davies clearly does not find absurd the idea that They have been here and that They are watching us without our knowing it. On the question of UFOs, he feels that "it is remarkable that so little scientific investigation of this important technological mythology has been undertaken."

Most of the book has a more sober aspect. Apart from jazzy chapter headings like "Stardoom" and "The Catastrophe Principle", Paul Davies has kept himself in check for the most part. You will have to read 70 pages of introduction to modern astronomy—the origin of the Solar System, galaxies, the big bang—before any mention of the beloved UFOs. I found these introductory chapters rather heavy going, but the reader unfamiliar with cosmology will probably welcome the thorough way that he explains the basic ideas of physics and astronomy.

After these introductory chapters we plunge into the question of "Life in the Universe" with critical faculties in abeyance. Straight away there is the assertion that "astronomers now conclude that there are billions of Earth-like planets in our galaxy alone". In fact there is no direct evidence for Earth-like or even Jupiter-like planets outside the Solar System. Then we come on to flying saucers, angels as spacemen, and Ezekiel's encounter with four flying wheels full of eyes. Personally I am more inclined to be-

lieve Ezekiel was on LSD than that he saw a flying saucer.

A more sober and conventional discussion of the question of interstellar communication follows. The idea that our civilisation is not unique is an ancient one, of course, going back to Lucretius and Giordano Bruno. Incidentally, Paul Davies is under the impression that Bruno was burnt for asserting the planets moved in ellipses. Of Bruno's many alleged heresies (including God's infinity implying an infinite universe; the motion of the earth; the stars being angels; that magic is a good thing; and on there being many worlds) elliptical orbits could hardly have been one. Kepler discovered them in 1609, some 9 years after Bruno's demise. While we are on the subject of history, it was Chéseaux, not Le Chatelier, who discussed the 'Olbers' paradox: Galileo was blinded by an inflammation of the eye in his last years, not by looking at the sun through a telescope (in his second letter to Marcus Welser on sunspots he describes very fully how to project the sun's image through a telescope onto a sheet of paper); and it was sunspots not solar granulation that he discovered.

The possibility of interstellar communication hinges on how long technological civilisations last. Paul Davies is confident that we have billions of years ahead of us, so that communication with other worlds and space travel become possible. Others may feel that the human race would be fortunate indeed to survive again the span that has elapsed since Lucretius.

Paul Davies then turns to the ultimate fate of the Universe. His chapter on the second law of thermodynamics and its implications is best in the book, and he explains very clearly how in an expanding universe order can grow out of chaotic initial conditions, but must ultimately decay. He outlines stellar evolution and the eventual fate of stars of different masses. The inevitable chapter on black holes follows, with an up-to-date account of the more esoteric "black hole theorems" and of the Hawking effect, in which there is a finite probability of a black hole emitting black body radiation (a significant effect for very low mass objects).

We are then back with the technology of billions of years hence—galactic empires, black hole energy sources, computer-dominated societies and the like. Paul Davies seems to think that modern technology was essential to make a country the size of the US into a nation state, but some notable empires of the past have managed with fairly simple technology. It does seem premature to worry about how mankind can prolong its existence

beyond the death of the Sun some billions of years hence. These speculations about the civilisation of the future are arid without some human or moral dimension. Even the pulpiest science fiction includes this.

According to current cosmological theory the Universe will either keep on expanding indefinitely, running down and getting colder, or it will start to contract after a while, collapsing to a second singularity, a big bang in reverse. In the former case Paul Davies holds out the vision of "a billion billion or more years of black hole manipulation following a mere one hundred billion years of uncontrolled stellar activity". He ends the book with a discussion of the steady-state theory and cyclical and time-reversing universes.

Most people will find the questions discussed in this book interesting, though they will not find much guidance in distinguishing between ideas based on empirical knowledge, ideas based on the extrapolation of theory, and pure fantasy. I did not find the book easy to read, but I suppose the ideas are more important than the prose. For me the most haunting idea

is that of the Universe running down and becoming cold and barren. As Byron describes it in *Darkness*:

I had a dream, which was not all a dream.
The bright sun was extinguished, and
the stars
Did wander darkling in the eternal
space,
Rayless, and pathless, and the icy earth
Swung blind and blackening in the
moonless air;

And this same vision is there in the opening of Konstantin's play in *The Seagull*, and in that sinister and desolate future seen by H. G. Wells in the last episode of *The Time Machine*.

And this brings us back to the question of the kind of popular science book that science itself needs. I am not sure that I am keen on these books that are an undifferentiated mixture of fact and fantasy, I prefer something more firmly grounded in the real world, or else a work of pure imagination like *The Time Machine* or *The Black Cloud*. But no doubt *The Runaway Universe* will be a great success.

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Guide to the Universe

A. M. Cruise

Cambridge Encyclopaedia of Astronomy. Edited by Simon Mitton. Pp. 481. (Jonathan Cape: London, 1977.) £15.

ALMOST every moment in the past fifty years has seemed opportune for the publication of an authoritative statement of Mankind's understanding of the Universe. The need to repeat the process is generated by new discoveries, which in astronomy are appearing ever more frequently, as are the publications. This book is one of the latest.

It is not an encyclopaedia in the normal sense in that it consists of twenty-three chapters on specific topics rather than an alphabetically ordered series of definitions and descriptions. It does, however, attempt to match the wide coverage of the subject to be expected in an encyclopaedia. The book was written by a team of sixteen specialists, mostly from the Institute of Astronomy at Cambridge, each contributing to the chapters within their speciality. Considerable effort has been expended in the organisation of the book and in the provision of tables of units, conversion factors and the definition of symbols. These should help the lay reader who is conscientious enough to keep referring to them. Any book that

calls itself an encyclopaedia and departs from the usual alphabetic ordering of topics must provide a good index, and some care has been taken in the organisation of the entries. Readers are directed to 'major descriptions' of topics by the provision of bold-type page numbers in the index and this is indicated in the text by the use of small capitals for the topic headings.

The first chapter serves as a very general introduction to the ideas involved in astronomy, in particular, the wide ranges of distance and scale encompassed by the various constituents of the Universe. It is clearly included in order to make the rest of the encyclopaedia accessible to interested readers without a formal scientific background. Another attempt to do this is made in an Appendix entitled "An Outline of Physics", which consists of a series of very brief notes, almost definitions in some cases, on various physical quantities and ideas. These sections expose the dilemma faced by the authors. How can one produce a readable and reasoned account of the current ideas in astronomy that will be both interesting and intelligible to people having a wide spectrum of previous knowledge? The style of writing in the text itself goes a long way to solving this problem. The chapters are on the whole both easy to read and lucid, even during the descriptions of potentially unfamiliar ideas such as electron degeneracy. It is a great pity therefore that the outline of physics was not included as a preliminary chapter using the same clarity of ex-

position evident elsewhere in the book.

Another facet of this dilemma concerns the use of mathematical equations in the text. This must be minimised for the popular market, although there is then the danger of much longer discussions in the text. The level at which mathematics is introduced is very variable throughout the encyclopaedia, perhaps reflecting the differing views of the contributing authors. A particularly bad example of this non-uniformity arises during the chapter on condensed states of matter where a concise derivation of the equation of state of a degenerate gas using three simple equations is almost immediately followed by an unnecessarily long and wordy argument explaining the relationship between the mass and radius of a white dwarf. Perhaps the elaborate avoidance of simple mathematics in popular books is creating a problem rather than solving one.

Even accepting the fact that astronomy started as a visual science, this book achieves an exceptionally high standard of visual presentation. The choice of photographs is excellent and there are some stunning full page (20 × 20 cm) colour pictures of nebulae. In almost every case the photographs are reproduced in a fairly large format which adds greatly to their impact and to the clarity of the evidence they provide. The diagrams are also very good and are a considerable aid to the text. The chapters on planets and those on galaxies benefit greatly from the choice of visual material and throughout the book this is one feature which will make the encyclopaedia stand out amongst competing volumes.

The introduction states the intention of emphasising firmly established new results, but one has the impression that there is a certain reluctance to discuss some of these new results in detail, perhaps to avoid the text being out dated. This tends to reduce the excitement of such a rapidly developing field of science. Surprisingly, the discussion of black holes falls into the same difficulty and this topic in particular might have benefited from the use of more visual material.

After very good descriptive chapters on stars, the Sun, the Solar System and interstellar material, the encyclopaedia reaches its climax with a series of four excellent chapters on galaxies. Again, the shrewd choice of illustrations gives a beautiful demonstration of the wide variety of galaxies and their interactions. Most readers will be disappointed with the coverage of cosmology which occupies a mere ten pages and is followed by a rather poor chapter on life in the Universe. These are both topics which fascinate anybody likely to pick up this book, and the treatment of them represents a serious error of judgement. The balance between these two chapters, and the rather extensive coverage of the

history of astronomy that follows, seems completely wrong. The final chapters describe the means by which measurements are made both from the ground and from space.

In addition to the appendix covering the basic physics used in the text, there are a series of star charts and information on how to use them. There is much description of how the star charts have been produced by computer, emphasising the accuracy achieved, although this accuracy

is far in excess of what can be utilised by anyone reading the charts. Perhaps this is an appropriate epilogue to this excellent semi-popular guide to the Universe. Judged against the authors' opinion of their work, expressed in the introduction, the encyclopaedia is sometimes disappointing. Judged against other books in the field it is often very good indeed. □

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Concise cosmology

Joseph Silk

Cosmology. By Michael Rowan-Robinson. Pp. 158. (Oxford University Press: Oxford, London and New York, 1977.). Hardback £6; paperback £2.95.

THE Universe offers a vast arena for observation and study. The modern science of cosmology comprises all that we observe of the large scale structure of the Universe, and much that we see on smaller scales. It blends observational data with a rich array of theoretical concepts, some of which are at the very forefront of physics. Yet it is an ancient discipline. Greek sages mused over topics that bear a strong similarity to those discussed by current pundits. A cynic might argue that this resurrection of ancient themes reinforces the notion of causality, a concept which is an intimate component of all models of the Universe, and indeed of modern physics. Some of the issues that have been vigorously debated by cosmologists over the past decade include such questions as: Did the early Universe emerge from chaos or from a relatively uniform initial state? How did the Universe attain its remarkable state of large scale regularity and isotropy? Were our atoms created in the initial instant of the big bang? What preceded the big bang? And was the development of intelligent life an occurrence unique to our Universe, or could other Universes have produced an environment suitable to the evolution of life?

To many astronomers and physicists, cosmology retains aspects of mythology. Few would deny that the boundary between physics and metaphysics becomes increasingly blurred as one probes more deeply into the nature of the singularity at the origin of the big bang. Yet modern cosmology is very much a "hard" science. The mysteries of the creation of the Universe have remained relatively sacrosanct, protected by the rigour of the mathematical formalism that pervades cos-

mology, and provides an effective deterrent against invasion by hordes of astrologers, mystics, clerics, and assorted seekers of truth.

Dr Rowan-Robinson's book *Cosmology* is a welcome attempt to penetrate the sophisticated barriers that have tended to make cosmology such an esoteric discipline in recent years. Although many of the more fundamental issues are not probed in this brief volume, it does serve as a useful introduction and survey of modern cosmology at an elementary level. The book is part of the Oxford Physics Series, ordinarily aimed at first-year mathematics and physics undergraduates, but it is at a level that anyone with a sufficiently good secondary school background in science should find accessible.

In reality, Dr Rowan-Robinson's book is an outline of cosmology. It is written in too concise a style to provide very much introductory or background material. Unavoidably perhaps, the coverage of modern astronomy is incomplete. Although some fields are covered, such as cosmic ray and neutrino astronomy, if only in a single page, other areas such as X-ray and gamma-ray astronomy receive little attention. A wide range of topics in cosmology and astrophysics is nevertheless encompassed. Noteworthy features are the discussions of the early stages of the big bang and of observational cosmology. These chapters fill a significant gap in that little has hitherto been available at a relatively elementary yet mildly mathematical level.

The author commences by summarising the available information obtained by astronomers over the entire electromagnetic spectrum. He describes the key properties of stars and galaxies and reviews the role of radio galaxies in cosmology. The empirical foundations of cosmology are thoroughly covered, including the redshift and background radiation. Big bang cosmology is discussed from the Newtonian and (schematically) from the general relativistic viewpoints. Final chapters are devoted to discussions of the mean density of matter in the Universe and of rival cosmological theories. The writing style is

lucid and the numerous illustrations are well integrated with the text.

Active researchers would consider this general approach to cosmology to be somewhat classical. It is in the spirit of Bondi's (by now historical) book of the same title, to which it provides a very useful modern supplement. Little mention is made of topics of current interest in cosmology, such as physical processes that occur near the singularity in the big bang models, and primordial black holes (perhaps deservedly) are not remarked upon. This book will serve as a useful initiation to cosmology for anyone desiring to delve into its more exotic aspects.

There are a number of, for the most part rather minor, points that this reviewer finds either misleading or erroneous. For example, the Newtonian swindle is performed in applying the Newtonian gravitation theory to cosmology, without mention of its justification. The key assumption is that the only force acting on a spherical shell is due to the matter within that shell. In fact, in an infinite Universe (as is required by the conventional big bang model), the gravitational potential energy of the shell would become arbitrarily large, and the force would not be well defined. The way around this apparent paradox is to make use of a theorem of general relativity (due to Birkhoff), from which one infers that space must be flat inside an empty cavity at the centre of a spherically symmetrical system. Only from this result can one justify the use of the Newtonian analogue that the gravitational force vanishes inside a uniform spherical shell of matter, and, consequently, that the contributions of all shells exterior to the one under consideration can be ignored.

Some specific points follow. On p88, it is a *non sequitur* to infer that the Universe was "probably very uniform" at decoupling, simply because the density was 1000 times the average density of matter within our galaxy. Only muon neutrinos and antineutrinos decouple at 10^{12} K, whereas electron neutrinos and antineutrinos decouple at 10^{10} K (p90). One omission is a crucial step in measuring the distance scale. This involves use of the period-luminosity relationship for Cepheid variable stars: only by knowing their absolute brightness can they be used as standard candles. The Lyman-alpha line is at 1216 Å, not at 912 Å (p120). Hydrogen molecules (p121) do not produce Lyman-alpha absorption lines but the Lyman and Werner absorption bands. Contrary to the author's statement, formation of globular-cluster-sized objects soon after recombination is not a serious difficulty for galaxy

formation, according to the conventional folklore that emanates from Princeton; these objects cluster together to form larger and larger systems, eventually identified with galaxies. Neutrino viscosity in the very early Universe is not considered by cosmologists to be responsible for significantly smoothing out inhomogeneity, although it may play a

role in isotropisation of the early Universe. The important processes that smooth out at least certain forms of inhomogeneity, leaving only galaxy- or cluster-sized fluctuations behind involve photon viscosity and diffusion, which occur at relatively late epochs.

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Lamb has shown that the 1970–72 period was more blocked than the 1900–50 mean, and Painting has demonstrated a decrease in blocking from 1965–69 to 1970–74. The statistical significance of these results is difficult to assess, and the whole area of climatic variability is one which requires a great deal more research. Subjective evaluations are worse than useless.

Gribbin's second main point is that global climate is significantly affected by changes in solar output, and that there have been such changes in the past. This is, in part, the old sunspot–climate story, a story which is closely related to the general question of climatic cycles. As an example of Gribbin's incomplete analysis, he discusses here the Kondratyev–Nikolsky relationship between sunspots and the solar constant, and Schneider and Mass's (guarded) use of this relationship. Both items are represented as being well established, yet they are not. The Kondratyev–Nikolsky relationship has always been open to doubt and its reality has recently (1976) been rejected completely by Paltridge and Platt. The simple Schneider–Mass model itself was never meant to be interpreted as proof of a solar–terrestrial link, but only as an indication that such a link was not totally unrealistic.

With climate cycles, *The Climatic Threat* defeats itself. On p.40, I find the statement that “there are no cycles” in climate “that are accepted as proven by everyone who works on climate problems”. Yet later, and in spite of this, is the irresponsible statement that “it seems quite reasonable to use the 180-year, 90-year and 22-year cycles” in solar activity “as well as the 11-year cycle, as a secure basis for climatic forecasting” (p115). If only things were so simple! In fact there is no proof that a 180-year cycle exists at all in either climate or solar activity; and the other cycles, although well established in the solar record, are uncertain and debatable in the climate record. Furthermore, even if these cycles in climate did exist, the key question, which Gribbin never asks, is how much of the variation in climate do they explain? It is fitting to close this review by returning to Cyril and Vivian.

Cyril: So you see, Vivian, how premature *The Climatic Threat* is. In climatology scientists are not so much revealing the order of Nature, as they are discovering Her complexities. They have delved successfully into many of these already, but there is much left to explore; and Nature delights in surprising those who underestimate Her.

The scene ends and Cyril and Vivian turn to exit as the first fingers of glacial ice creep under the french windows. □

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Climatic change

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The Climatic Threat: What's Wrong with our Weather? By John Gribbin. Pp. 206. (Fontana: London, 1978.) Paperback £1.

IN his dialogue *The Decay of Lying* between the characters Cyril and Vivian, Oscar Wilde gave us a marvellous aphorism which can be used to describe many recent pseudoscientific works: “. . . facts are either kept in their proper subordinate position, or else entirely excluded on the general ground of dullness”. It would be harsh of me to describe John Gribbin's book in this way; his work is much more subtle. Nevertheless, it might be entertaining to listen in to Cyril and Vivian for a little while. The scene is the library of a country house somewhere in England.

Cyril (coming in through the open window from the terrace): My dear Vivian, don't coop yourself up all day in the library. It is a perfectly lovely afternoon. Let us go and lie by the river and enjoy Nature.

Vivian: Enjoy Nature! My own experience is that the more we study science, the more we fear Nature. Science reveals to us Nature's love of design, the inexorable inevitability and predictability of Her changes of mood; and our vulnerability to them.

Cyril: Ah! You have been reading *The Climatic Threat* again I see. Well might you stay inside if you believe that tale. But I, for one, do not; though in truth I feel others may. Man is so eager to believe the impossible! Such plausible expositions are more tempting than apples to Eve.

. . . which gives me the opportunity to take over from Cyril and review *The Climatic Threat*. The book is a personal evaluation of some recent research into the processes of climatic change, and goes so far as to present forecasts of future climate which accord with this evaluation. At the beginning the author warns the reader that his book is subjective; that he “throws caution to the winds”. The book is, however, well argued (at least superficially), and is written in an objective and authoritative manner. Unfortunately, as Cyril well realises, this style can only deceive the “average reader”, who cannot possibly know how incompletely the author reviews the fields he discusses, how uncritical and selective are his references to the scientific literature, how much he

has mixed sound well accepted work with controversial opinion and speculation, and how often the cautious, tentative words of others are represented as established fact. By presenting such an incomplete “subjective” (that is, “biased”) survey, and by quoting uncritically the predictions of future climatic changes given by others (for example, Willett), Gribbin gives the impression that the processes of climatic change are well understood. They are not; in fact even our knowledge of how climate has changed in the past is sketchy and uncertain. For example, again contrary to the impression which Gribbin creates, climatologists do not even know how global temperature has changed over the past 100 years, let alone how it has varied over the past 1000 or more years. There are vast areas of the globe, especially in the southern hemisphere, where the observational data network is so sparse that the magnitude and direction of temperature changes in those regions are virtually unknown.

The Climatic Threat weaves its story around two main hypotheses. The first of these concerns the link between climatic variability and the general circulation of the atmosphere; the second involves the link between changes in the Sun and changes in the Earth's climate. First, Gribbin explores the hypothesis that global (or perhaps it is Arctic; Gribbin is somewhat ambiguous on this point) cooling leads to a weaker, more meridional circulation pattern, with attendant increases in the frequency of blocking situations (such as occurred during the 1976 European drought) and in the year to year variability in climate. This is an attractive hypothesis, but it is based on a number of assumptions which have yet to be tested. Furthermore, despite Gribbin's contention that the climate has become more variable recently, there has been no comprehensive statistical analysis of recent data to show whether or not significant changes in climatic variability and/or the frequency of extreme events have occurred in recent decades. Most statements made both by Gribbin and by other authors on this question lose statistical significance because they are made *a posteriori*, soon after some extreme event, and so are temporally selective. As far as changes in the frequency of blocking are concerned, available evidence (not mentioned by Gribbin) is incomplete and equivocal.

articles

Seismic evidence for sedimentary troughs of Mesozoic age on the Hebridean continental margin

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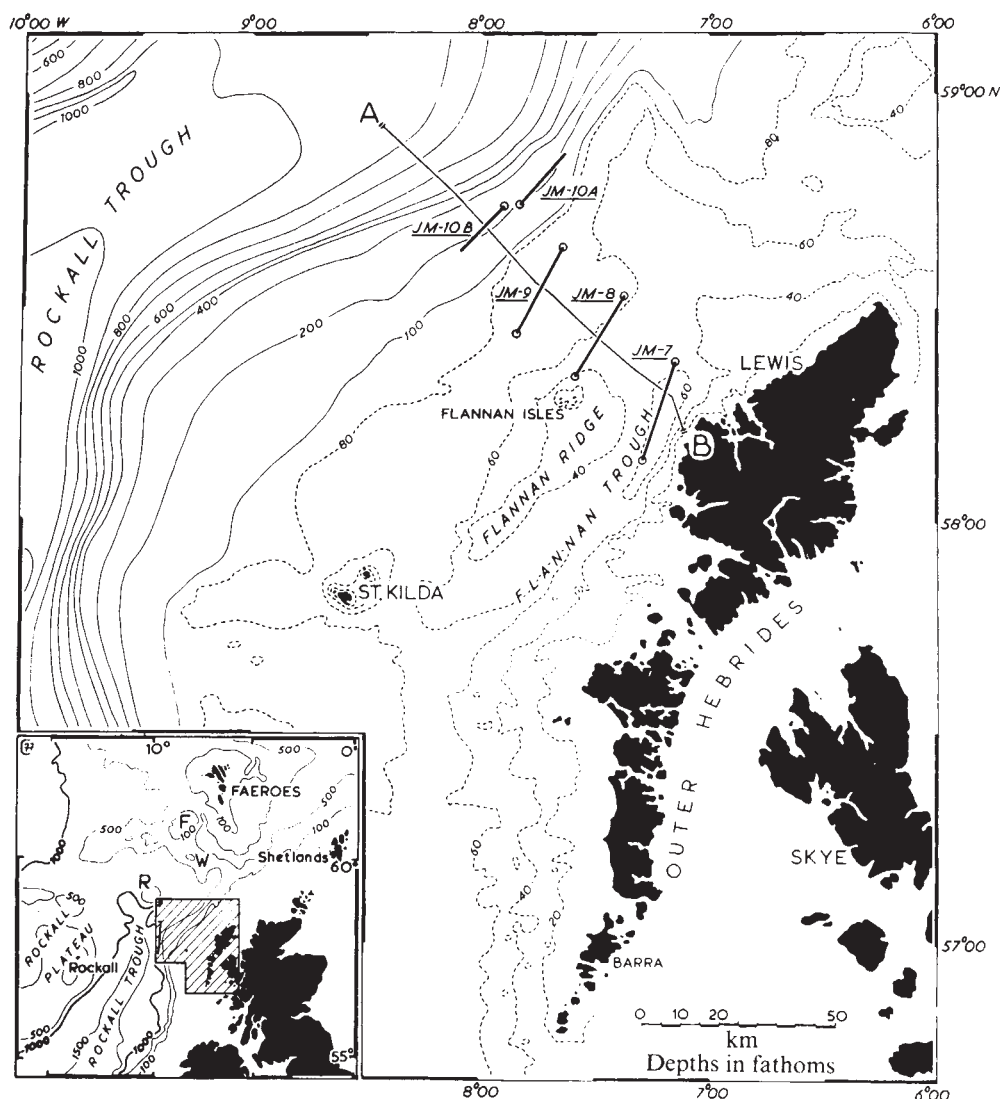
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Seismic refraction shooting near the Isle of Lewis in the Outer Hebrides has shown the presence of major sedimentary units. Their age is estimated from seismic velocities and reveals important data on the structural evolution and petroleum potential of the Rockall Trough and its environs.

THE history of sedimentation on the continental shelf and slope west of the Outer Hebrides is of considerable interest because of its bearing on the structural evolution and petroleum

potential of the Rockall Trough and its environs (Fig. 1). At present, however, there are many uncertainties in interpreting the sedimentary record because the distribution of the sediment cover is poorly known and the stratigraphical control is limited to only a small number of core and dredge samples from the Rockall Trough¹⁻³ and to inferences based on the configuration of seismic reflectors⁴⁻⁷. To provide a clearer picture of the pattern of Mesozoic and Tertiary sedimentation on the Hebridean margin, a programme of seismic refraction shooting

Fig. 1 Bathymetry of the Hebridean continental margin in the vicinity of seismic refraction profiles JM-7 to JM-10B (20 fm = 36.6 m). Positions of sonobuoys on each line are indicated by the small circles. Magnetic and gravity anomalies along line A-B are shown in Fig. 3. F, Faeroe Bank; W, Wyville Thomson Ridge; R, Rosemary Bank.



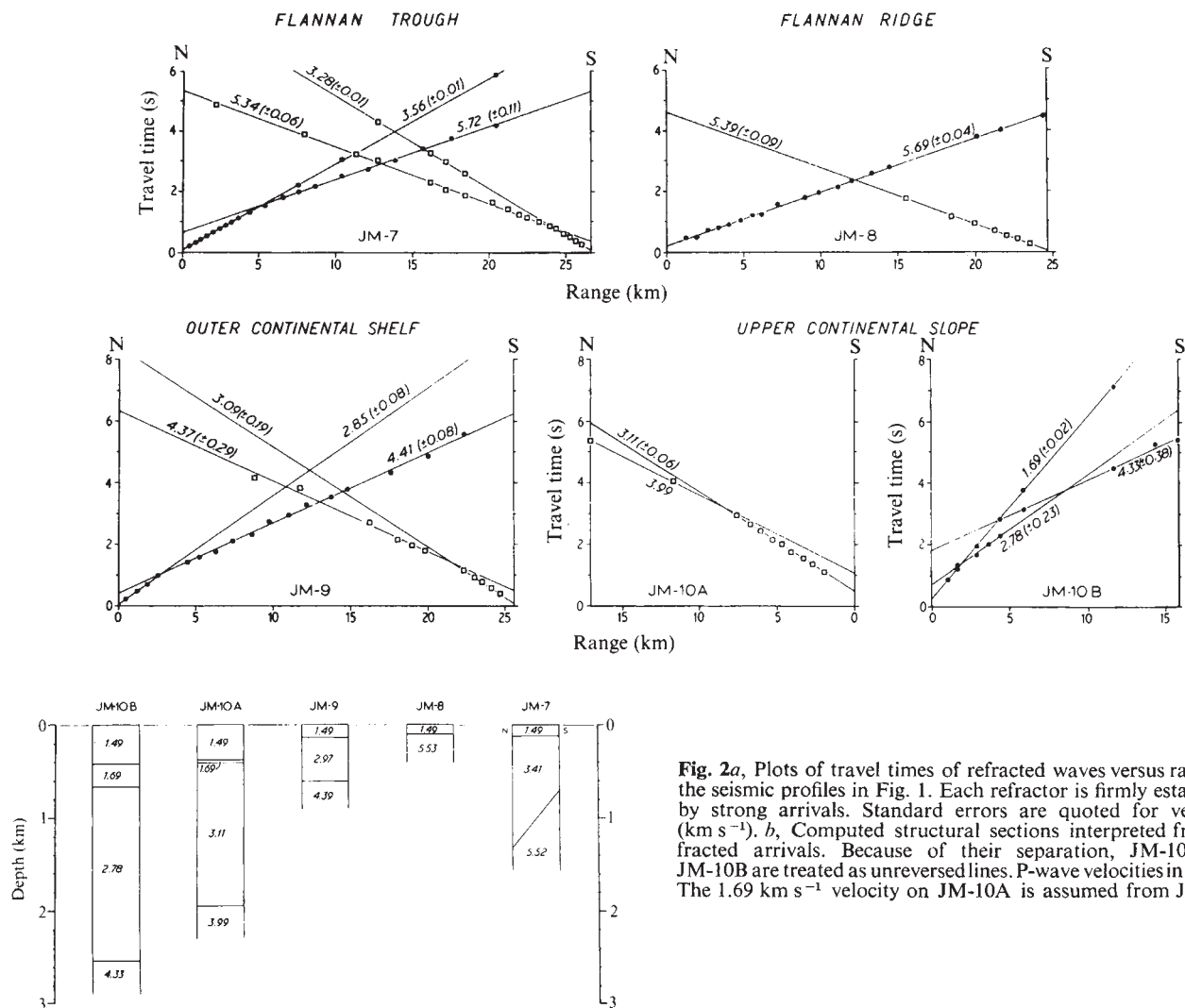


Fig. 2a, Plots of travel times of refracted waves versus range for the seismic profiles in Fig. 1. Each refractor is firmly established by strong arrivals. Standard errors are quoted for velocities (km s^{-1}). b, Computed structural sections interpreted from refracted arrivals. Because of their separation, JM-10A and JM-10B are treated as unreversed lines. P-wave velocities in km s^{-1} . The 1.69 km s^{-1} velocity on JM-10A is assumed from JM-10B.

was carried out from RRS John Murray during summer 1975. Here five profiles shot with conventional explosive charges and sonobuoys near the Isle of Lewis are described. These lines reveal the presence of several major sedimentary units, the ages of which can be approximately determined from the seismic velocities. Structural sections computed from the observed refracted arrivals are presented in Fig. 2.

Indications of at least one sedimentary layer are found except along profile JM-8, where a high velocity refractor (5.53 km s^{-1} ; Fig. 2) occurs at the seabed. As the velocity on this line is closely similar to the values characteristic of the quartz-feldspathic gneisses of the Lewisian⁸, the 5.53 km s^{-1} material is interpreted as the continuation of the basement rocks on the Flannan Isles (Fig. 1) which have been described by Stewart⁹. The 110 m-deep depression—here called the Flannan Trough (Fig. 1)—between Lewis and the north-east trending ridge on which the Flannan Isles lie, is underlain by a 3.41 km s^{-1} layer that thickens northwards (JM-7; Fig. 2). The 5.52 km s^{-1} refractor beneath can be correlated with the Lewisian basement of the Flannan Ridge and of Lewis itself^{10,11}. Layers with seismic velocities of 2.97 km s^{-1} and 4.39 km s^{-1} have been detected on profile JM-9. The upper can be considered equivalent to the $2.78\text{--}3.11 \text{ km s}^{-1}$ unit on lines JM-10A and JM-10B, while the 4.39 km s^{-1} material can be correlated with the $3.99\text{--}4.33 \text{ km s}^{-1}$ layer (Fig. 2). The $2.78\text{--}3.11 \text{ km s}^{-1}$ section clearly thickens rapidly to the north-west. On the continental slope, it is covered by a 1.69 km s^{-1} layer. Refractions from below the $3.99\text{--}4.39 \text{ km s}^{-1}$ material were not recorded. If it is underlain by 5.5 km s^{-1} basement, its minimum thicknesses at JM-9 and JM-10B, which can be computed by assuming that the basement begins to produce first arrivals

at the range of the furthest shots, are 3.8 km and 1.9 km , respectively. At JM-10A the minimum layer thickness is 2.8 km .

Seismic boundaries and structural features

Seismic boundaries have been interpolated between the refraction stations using a gravity profile over the shelf portion of line A-B (Figs 1, 3). In deriving the section in Fig. 3 it has been assumed that the basement has a uniform density of 2.65 g cm^{-3} and extends to a depth of 26 km landwards of the shelf break. An apparently undistorted Moho at this depth has been detected by Smith and Bott¹² in a similar structural setting west of the Shetlands. Local Airy compensation is assumed beneath the Hebridean continental slope. Although the resulting interpolations are necessarily approximate, they reveal some important structural features. For example, the steepness of the Lewisian surface near kilometres 70 and 85 indicates that the Flannan Ridge is flanked by faults. Likewise, the presence of basement rocks along the coast of Lewis requires a steep, probably faulted, contact only a few kilometres offshore. The Flannan Trough is clearly an asymmetrical structure, most of it being magnetically quiet (Fig. 3). Notable in this region is the major discrepancy between the observed gravity anomaly and that calculated using the refraction data. With the density contrast of 0.32 g cm^{-3} obtained from the seismic velocities, the sediment thickness at JM-7 is computed to be 1.8 km , whereas the refraction line gives a thickness of only 1.0 km . This difference implies that significant density contrasts must occur within the Lewisian. More refined structural models of the basement will be given elsewhere.

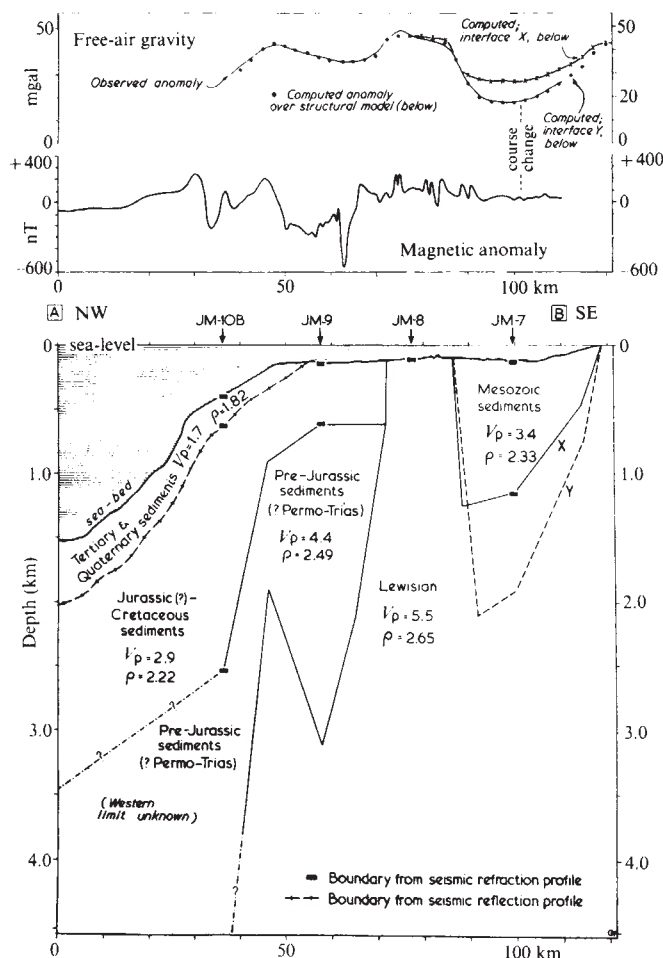
North-west of the Flannan Isles, in a region encompassing part of the major crustal transition between the continental

shelf and the Rockall Trough, lies a second sedimentary basin which contains 4.4 km s^{-1} material in addition to a lower velocity section. Without a knowledge of deep crustal structure and with such limited seismic coverage of the shallow structural features, the configuration of the seismic boundaries over this section cannot yet be determined in detail. Nevertheless, it is evident from the present gravity profile and from the constraints imposed on layer thicknesses along the seismic lines, that the 4.4 km s^{-1} unit under the shelf is thickest in the vicinity of JM-9. Furthermore, the layer seems to thicken north-westwards from the outermost continental shelf. The long wavelength negative magnetic anomaly between kilometres 45 and 70 is apparently associated with the depressed Lewisian surface.

Seismic layering

The seismic layering in Fig. 2 can be broadly interpreted in stratigraphic terms (Fig. 3). On profile JM-10B, the shallowest unit, the base of which coincides with a strongly reflective unconformity observed on an airgun profile along line A-B (Fig. 1), is inferred to be of Tertiary-Quaternary age as its velocity lies within the range characteristic of post-Cretaceous successions beneath the north-west European shelf (1.6 – 2.25 km s^{-1} ; refs 13, 14). Velocities between 2.25 km s^{-1} and the basement value of 5.5 km s^{-1} , must be interpreted with some caution as this is a marginal area where changes in thickness are rapid and large facies variations are likely to be found. Although common in Mesozoic sections, the values of 2.78 – 3.41 km s^{-1} in Fig. 2 cannot unequivocally distinguish Cretaceous from Jurassic sediments or Triassic shales and marls¹⁴.

Fig. 3 Structure of the Hebridean margin along line A-B in Fig. 1. Densities are derived from seismic velocities using the Nafe-Drake curve²⁶. ρ in g cm^{-3} ; V_p in km s^{-1} .



Lying below a probable Tertiary sequence on the continental slope, the 2.78 – 3.11 km s^{-1} layer is tentatively interpreted here as being mostly of Cretaceous age. Senonian argillaceous limestone has been recovered from below a prism of Tertiary sediments to the west of Ireland¹ and Maestrichtian chalk has been dredged from the Anton Dohrn seamount², but no Jurassic or Triassic sediments have been reported from this region.

The infilling of the Flannan Trough has an appreciably higher velocity than the uppermost layer at JM-9 (Fig. 2), suggesting that it is somewhat older, although facies variations cannot be eliminated as the sole cause of the increased velocity. 3.41 km s^{-1} is close to the average seismic velocities of several Jurassic sequences around Britain^{15–17}, but it does not exclude the presence of Cretaceous or Triassic sediments immediately west of Lewis. The infilling is thus designated as undivided Mesozoic in Fig. 3.

The distribution of the 4.4 km s^{-1} material near JM-9 (Fig. 3) suggests that it is sedimentary, the high velocity indicating a pre-Jurassic age. Torridonian and Devonian sediments, although possible constituents of this layer, are not considered likely because published velocity determinations (ref. 18) and unpublished data on land indicate that their average seismic velocities are generally greater than 4.8 – 5.0 km s^{-1} . In view of the presence of 3 km -thick New Red Sandstone sequences within a major fault-bounded trough in the Minch^{19,20}, the 4.4 km s^{-1} layer is probably late Palaeozoic-Triassic in age. This interpretation is supported by recent sections of Cashion²¹, based on commercial seismic and well data, showing Jurassic-Cretaceous sediments resting on thick Triassic deposits in a graben west of the Shetlands, which Bott and Watts²² first postulated to be of Mesozoic age from gravity data.

Discussion

The occurrence of thick Mesozoic sediments west of the Outer Hebrides and the absence of a seismically detectable Tertiary-Quaternary sediment cover over most of the shelf have important implications when we consider the evolution of the continental margin and the Rockall Trough. Mesozoic sedimentation to the north-west of Lewis was clearly controlled by subsidence along four major fault zones, indicated by the steep boundaries near kilometres 45, 70, 85 and 120 in Fig. 3. Areas affected by the vertical movements, which exceed 4 km under the upper continental slope, became tectonically stable in the Tertiary when the present continental shelf appears to have formed a shallow or emergent platform on which little sediment accumulated. The seismic evidence for a substantial thickness of pre-Jurassic sediments north-west of the Flannan Isles suggests that the Rockall Trough has a history of tectonic movements extending back into Triassic or before. As the 3.99 – 4.39 km s^{-1} section seems to expand rapidly just below the shelf break (Fig. 3), this latter region has probably been a major structural boundary since the early Mesozoic or late Palaeozoic. Judging by the similarities in seismic velocities, the trough beneath the outer continental shelf probably developed at about the same time as that under the continental slope. The Hatton-Rockall basin on the Rockall Plateau²³ may also have been formed in response to the same regional stress pattern and may therefore contain pre-Jurassic sediments.

Interpretation of structural evolution of the region greatly depends on the limit of the pre-Jurassic section west of JM-10B, which is at present unknown. Two models may be proposed. In the first, the Rockall Trough subsides in late Palaeozoic or Triassic time, possibly in association with the generation of oceanic crust in its central zone. Pre-Jurassic sediments extend from the Hebridean slope to the eastern flank of the Rockall Plateau. In an alternative model, pre-Jurassic deposits are confined to areas outside the magnetically quiet zone typical of much of the Rockall Trough. The latter develops by seafloor spreading in Jurassic or Cretaceous time, following a long period of downwarping near its present margins that started in the late Palaeozoic or Triassic. As many pre-Jurassic sediments

have seismic velocities overlapping values reported for basement in the Rockall Trough^{24,25}, drilling rather than seismic methods will probably be necessary to resolve their distribution. Despite the present ambiguities, however, the occurrence on the Hebridean margin of 2 km of Upper Mesozoic and Tertiary sediments overlying a thick accumulation which is probably pre-Jurassic in age is of importance in relation to current exploration for petroleum on the western side of Britain. The thick pre-Tertiary sequences may contain valuable source rocks for petroleum, while faulting associated with Mesozoic subsidence may have produced significant structural and stratigraphic traps.

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Mechanism of resistance to thiostrepton in the producing-organism *Streptomyces azureus*

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An antibiotic-producing organism, Streptomyces azureus, defends itself against its own toxic product by methylating the RNA of its ribosomes.

THE antibiotic, thiostrepton, inhibits protein synthesis in prokaryotic, but not in eukaryotic cell-free systems. However, various Gram-negative organisms, including *Escherichia coli*, are impermeable to the drug and are, therefore, resistant to it *in vivo*. One notable exception is *Streptomyces azureus*, the organism which produces thiostrepton. When grown on agar plates, *S. azureus* is sensitive to a range of antibiotics which act on bacterial ribosomes, but it is resistant to thiostrepton (and to siomycin, sporangiomycin and thiopeptin, compounds which have identical modes of action to that of thiostrepton)¹. Although reproducibly active cell-free protein-synthesising systems from *Streptomyces* have not been prepared, it has previously been shown that ribosomes from *S. azureus* bind ³⁵S-thiostrepton poorly whereas those from *S. coelicolor* and other *Streptomyces* sp. bind the drug with approximately 1:1 stoichiometry¹ (Table 1). We show here that resistance to thiostrepton in ribosomes of *S. azureus* is determined by the properties of the 23S ribosomal RNA. Furthermore, *S. azureus* possesses a methylase which acts on the RNA of other bacterial ribosomes and renders them resistant to thiostrepton.

Binding of ³⁵S-thiostrepton to ribosomal subunits and core particles

When a mixture of 50S and 30S subunits derived from *E. coli* ribosomes was treated with ³⁵S-thiostrepton at 3 mM Mg²⁺ and centrifuged into a sucrose density-gradient, the drug bound exclusively to the heavier (50S) particles (see Fig. 1). In other experiments, similar observations were made using ribosomal subunits from *S. coelicolor*. Accordingly, in the following experiments, it is assumed that binding of thiostrepton occurs only on 50S subunits or on various particles derived

from them. The validity of this assumption is supported by observations in other laboratories^{2,3}.

When ribosomes or their subunits are exposed to high concentrations of LiCl, they shed various surface proteins

Table 1 Binding of ³⁵S-thiostrepton to ribosomal core particles and partially reconstituted particles

Cores (70-75 pmol)	³⁵ S-thiostrepton bound (pmol)	Split proteins used in partial reconstitution	³⁵ S-thiostrepton bound after partial reconstitution (pmol)
<i>S. coel.</i> 1Mc	5	<i>S. coel.</i> 1Msp.	54
<i>S. coel.</i> 1Mc	5	<i>S. az.</i> 1Msp.	51
<i>S. coel.</i> 4Mc	0	<i>S. coel.</i> 4Msp.	50
<i>S. coel.</i> 4Mc	0	<i>S. az.</i> 4Msp.	48
<i>S. coel.</i> 6Mc	0	<i>S. coel.</i> 6Msp.	53
<i>S. coel.</i> 6Mc	0	<i>S. az.</i> 6Msp.	55
<i>S. az.</i> 2Mc	0	<i>S. coel.</i> 2Msp.	2
<i>S. az.</i> 2Mc	0	<i>S. az.</i> 2Msp.	2
<i>S. az.</i> 6Mc	0	<i>S. coel.</i> 6Msp.	1
<i>S. az.</i> 6Mc	0	<i>S. az.</i> 6Msp.	2
Control ribosomes for comparison			
<i>S. coel.</i> 70S	65	—	—
<i>S. az.</i> 70S	2	—	—

Core particles(c) and split proteins(sp.) were prepared from 70S¹ ribosomes according to the methods of Nierhaus and Montejó⁶ except that exposure to LiCl (1.2,4.6M) was in the presence of 1 mM MgCl₂. Cores were washed by recentrifugation in TMAß buffer and were stored in this buffer at -70 °C. Split proteins were concentrated by dialysis against TMAß buffer containing 20% w/v Carbowax and were stored at -70 °C in TMAß buffer at approximately 4 pmol equivalents µl⁻¹. A pmol equivalent of split proteins was defined as those obtained from 1 pmol 70S ribosomes. Partial reconstitution was carried out at 0 °C for 10 min by adding 2-3 equivalents of splits to a given amount of cores. ³⁵S-thiostrepton (70 pmol) was then added and incubation continued at room temperature for 10 min before samples were taken for gel filtration to separate ribosome-bound drug from free drug as previously described¹. The specific radioactivity of ³⁵S-thiostrepton varied over 280-210 c.p.m. per pmol during these experiments.

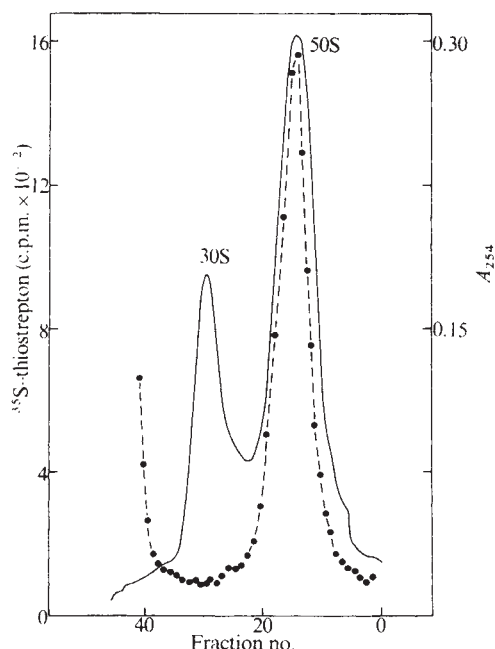


Fig. 1 Binding of ^{35}S -thiostrepton to *E. coli* ribosomal subunits. A mixture of 50S and 30S ribosomal subunits of *E. coli* ($5 A_{260}$ units) in buffer containing 10 mM Tris. HCl (pH 7.6), 3 mM MgCl_2 , 50 mM NH_4Cl , 3 mM β -mercaptoethanol (TMA β buffer) was incubated at 20 °C for 10 min with ^{35}S -thiostrepton (125 c.p.m. pmol^{-1} ; 70 pmol in 5 μl of dimethyl sulphoxide). The incubation mixture was then layered on to a sucrose density-gradient (15–30% sucrose in TMA β buffer, total volume 5.0 ml) and centrifuged at 40,000 r.p.m. for 270 min at 2 °C in the Spinco SW 50.1 rotor. After centrifugation, the absorbance of components in the gradient was monitored continuously at 254 nm in an Isco DUA-2 gradient analyser, and the emergent liquid was collected as a series of fractions into scintillation fluid for estimation of their radioactivity.

(‘split proteins’) and yield ribonucleoprotein ‘core particles’. These can be derived from either subunit and contain 23S RNA (from the 50S subunit) or 16S RNA (from the 30S subunit). The exact protein content of a core particle is determined by the salt concentrations used in its production.

Cores (‘1M cores’) produced from *E. coli* 50S ribosomal subunits in 1M LiCl do not bind thiostrepton⁴, nor do similar particles produced from 70S ribosomes of *Bacillus megaterium*⁵ or *S. coelicolor* (Table 1). In each case, the ability to bind thiostrepton can be restored by incubating cores with split proteins after removal of LiCl. As shown in Table 1, various split-protein preparations from either *S. coelicolor* or *S. azureus*

were able to restore the ability to bind thiostrepton to a range of *S. coelicolor* cores, whereas cores derived from *S. azureus* could not be ‘activated’ for drug binding regardless of the source of the split proteins used. Clearly, some component of the 6M cores of *S. azureus* ribosomes is responsible for resistance to thiostrepton in this system, and as such cores contain very few ribosomal proteins, it was decided to examine the ribosomal RNA (rRNA) directly.

Total reconstitution of ‘hybrid’ 50S ribosomal subunits

Attempts to reconstitute active 50S ribosomal subunits often fail, perhaps due to inactivation of one or more ribosomal components during the heating step(s) commonly used (see, however, ref. 7). One elegant way of avoiding this problem came from Nomura *et al.*⁸ who first reconstituted 50S subunits starting with RNA and proteins derived from ribosomes of *B. stearothermophilus*, a thermophile which grows optimally at 65 °C. Subsequently, active 50S particles were produced containing rRNA from various sources and ribosomal proteins (r proteins) from *B. stearothermophilus*⁹. Using similar techniques (Table 2), hybrid 50S particles were created containing rRNA from *S. azureus* or *S. coelicolor*, with r proteins from *B. stearothermophilus* 50S subunits, and their response to thiostrepton was examined.

Hybrid particles containing rRNA from *S. coelicolor* bound ^{35}S -thiostrepton stoichiometrically (1:1), whereas those containing *S. azureus* rRNA bound much less of the drug (Table 2). However, both types of hybrid 50S particle were active in supporting the formation of [ribosome–EF G–guanine nucleotide] complexes in the presence of fusidic acid. This reaction is known to occur on the 50S ribosomal subunit¹¹ and is specifically inhibited by thiostrepton but not by any of the many other antibiotics known to bind to the 50S subunit (such as chloramphenicol, lincomycin, sparsomycin)^{12,13}. The formation of complexes of this type mimics one of the partial reactions of protein synthesis (the ‘translocation’ reaction) during which the protein EF G binds to, and later dissociates from, the 50S ribosomal subunit, and GTP is hydrolysed. As some of the hybrid particles were only feebly active in protein synthesis in appropriately supplemented systems (data not shown), the formation of [ribosome–EF G–guanine nucleotide] complexes was used as an assay for the functional reconstitution of that region of the 50S ribosomal subunit to which thiostrepton normally binds. From Table 2, it can be seen that both types of hybrid were active in supporting complex formation (activity 20–25% that of unfractionated 50S subunits of *B. stearothermophilus*) with purified EF G protein derived from *E. coli*. Table 2 also shows that, whereas hybrid particles containing *S. coelicolor* rRNA were sensitive to the action of

Table 2 Response of totally reconstituted particles to thiostrepton

Particles constructed (40 pmol)		pmol ^{35}S -thiostrepton bound	^3H -guanine nucleotide retained on filters c.p.m. minus blank (700 c.p.m.)		
Source of rRNA	Source of r Protein		Control	+ 280 pmol thiostrepton	+ 1.4 nmol thiostrepton
<i>B. stearo.</i> 50S	<i>B. stearo.</i> 50S	30	13,460 (100%)	—	zero
<i>S. az.</i> 70S	<i>B. stearo.</i> 50S	9	17,060 (100%)	12,280 (72%)	7,170 (42%)
<i>S. coel.</i> 70S	<i>B. stearo.</i> 50S	42	13,560 (100%)	4,340 (32%)	950 (7%)
<i>S. coel.</i> 3M cores methylated	<i>B. stearo.</i> 50S	10	9,100 (100%)	—	7,010 (77%)
Standard particles (unfractionated)					
<i>B. stearo.</i> 50S	40 pmol	30–35	69,180 (100%)	12,634 (18%)	6,816 (10%)
<i>E. coli.</i> 70S	40 pmol	35–40	84,520 (100%)	32,120 (38%)	6,761 (8%)

70S ribosomes from *S. azureus* and *S. coelicolor* and 50S subunits from *B. stearothermophilus* were dissociated in LiCl and urea to yield RNA and protein fractions according to Nomura and Erdmann⁸. The RNA fraction was then freed of tightly bound ribosomal protein L3 according to Fahnestock *et al.*¹⁰ RNA 70 (from 70S ribosomes), RNA 50 and Prot 50 (from 50S subunits) and L3 were used without further purification in the buffers specified by Fahnestock *et al.*¹⁰. One A_{260} equivalent of protein was defined as that amount of protein derived from one A_{260} unit of the corresponding ribosomal particles. Reconstitution mixtures contained (per ml): 15 A_{260} units of RNA 70 or RNA 50 (approximately 150 μl), 15 A_{260} equivalents of *B. stearo.* Prot 50 (75 μl), 16 A_{260} equivalents of *B. stearo.* L3 (100 μl) and reconstitution buffer supplemented with 1M MgCl_2 to bring the final Mg^{2+} concentration to 20 mM. Reconstitution buffer contained: 30 mM Tris. HCl (pH 7.6), 20 mM MgCl_2 , 300 mM KCl, 6 mM β -mercaptoethanol. The mixture was heated at 55 °C for 4 h, dialysed at 0 °C against TMA β buffer containing 10mM MgCl_2 and stored as aliquots at –70 °C. Reconstituted particles were assayed for binding of ^{35}S -thiostrepton (THS) and for formation of [ribosome–EF G–guanine nucleotide] complexes as previously described¹. Protein elongation factor G (EF G) was from *E. coli*.

Table 3 Binding of ^{35}S -thiostrepton to totally reconstituted particles containing *Streptomyces* ribosomal RNA and proteins

Particles assayed (30 pmol)		pmol ^{35}S -thiostrepton bound (510 c.p.m. per pmol)
rRNA source	r Protein source	
<i>S. coel.</i> 70S	<i>S. coel.</i> 70S	14.5
<i>S. coel.</i> 70S	<i>S. az.</i> 70S	14.0
<i>S. az.</i> 70S	<i>S. az.</i> 70S	0.5
<i>S. az.</i> 70S	<i>S. coel.</i> 70S	0

Ribosomes (70S) from both *S. coelicolor* and *S. azureus* were taken apart in LiCl + urea as in Table 2 to yield RNA 70 and Prot 70 preparations. Unlike the experiments described in Table 2, protein L3 was not removed from RNA preparations. Reconstitution was then carried out using RNA 70 and Prot 70 from these two *Streptomyces* essentially as in Table 2. Binding of ^{35}S -thiostrepton to reconstituted particles was assayed as described elsewhere¹.

thiostrepton, those containing rRNA from *S. azureus* were much more resistant to the drug.

As the protein input in reconstitution incubations was derived exclusively from the 50S subunits of *B. stearothermophilus* ribosomes which are sensitive to thiostrepton (Table 2), it is suggested that some property of the RNA of the 50S subunit is responsible for the resistance to thiostrepton of *S. azureus* ribosomes and of hybrids containing *S. azureus* rRNA. Further, as 5S RNA separates largely into the 'protein' fraction when ribosomes are extracted with LiCl and urea⁸, any 5S RNA in the hybrid 50S particles would be derived largely from *B. stearothermophilus*. Accordingly, it is suggested that the 23S RNA of the *S. azureus* ribosome determines resistance to thiostrepton.

Particles were also reconstituted containing rRNA and ribosomal proteins from *S. azureus* and *S. coelicolor* in all four possible combinations (Table 3). None of the resultant particles was active in protein synthesis and they were only weakly active in the formation of [ribosome-EF G-guanine nucleotide] complexes. Clearly, the optimal conditions for formation of such particles differ from those actually used. Again, however, regardless of the source of the ribosomal proteins used, particles containing *S. coelicolor* rRNA bound ^{35}S -thiostrepton, whereas those containing *S. azureus* rRNA did not. These experiments indicate that the ribosomal proteins of *S. azureus* are fully capable of forming a binding site for thiostrepton in appropriate conditions, and again implicate *S. azureus* rRNA as the determinant of resistance to thiostrepton in that organism.

Methylation of ribosomal RNA in *S. azureus*

Clearly, 23S ribosomal RNA can, in association with a given complement of ribosomal proteins, order the structure of the 50S ribosomal subunit so as either to promote (*S. coelicolor* rRNA) or prevent (*S. azureus* rRNA) the binding of thio-

strepton without affecting the activity of that subunit in protein synthesis. In principle, the crucial difference between the two species of 23S RNA might be due to differences in their nucleotide sequences or in post-transcriptional modification patterns or in both. Previously, when drug resistance had been shown to be determined by rRNA (refs 14, 15), the state of methylation of the rRNA (either over-methylation¹⁵ or under-methylation¹⁴) had been the determining factor. Therefore, extracts of *S. azureus* and *S. coelicolor* were examined for a methylase activity capable of altering the response of a ribosome to thiostrepton.

Extracts of *S. coelicolor* apparently do not possess any enzyme activity (methylation, acetylation or phosphorylation) capable of rendering *S. azureus* ribosomal particles sensitive to thiostrepton (data not shown). However, among those proteins removed from *S. azureus* ribosomes by washing in buffers containing 1 M NH_4Cl , methylase activity which renders *S. coelicolor* ribosomal particles resistant to thiostrepton was detected (Table 4). This crude methylase does not affect the binding of ^{35}S -thiostrepton to intact 70S ribosomes of either *S. coelicolor* (Table 4) or *B. megaterium* (data not shown). However, when core particles from *S. coelicolor* ribosomes were incubated with the crude methylase (with *S*-adenosyl-methionine as methyl donor) and appropriate split proteins then added, the resultant particles bound ^{35}S -thiostrepton poorly in comparison with controls. That this effect is specific and not due to random, uncontrolled methylation occurring in *in vitro* conditions is also indicated in Table 4; a ribosomal wash from *S. coelicolor* which also possessed methylase activity (data not shown) did not alter the ability of *S. coelicolor* 1 M cores to accept 1 M split proteins and then to bind ^{35}S -thiostrepton.

In other experiments (data not presented), methylation of *S. coelicolor* core particles was carried out using crude methylase from *S. azureus* with [methyl- ^3H]-*S*-adenosyl-methionine as cofactor. When methylated cores were digested with trypsin or with a combination of T1 and pancreatic ribonucleases or with hot trichloroacetic acid, it was clear that rRNA and not ribosomal protein was methylated. This point was also established in an experiment designed to counter the possibility that methylation of *S. coelicolor* cores by *S. azureus* crude methylase (as in Table 4) impaired their ability to re-accept split proteins, rather than their ability to bind thiostrepton *per se*. Thus, RNA was extracted from 3 M cores of *S. coelicolor* following methylation with *S. azureus* crude methylase and *S*-adenosyl-methionine (as in Table 4) and used to prepare hybrid particles with 50S ribosomal proteins from *B. stearothermophilus*. As shown in Table 2, the resultant hybrids were unable to bind ^{35}S -thiostrepton and were resistant to the drug in the functional assay.

Conclusions

S. azureus protects itself against its own toxic product, thiostrepton, by methylating the 23S RNA of its 50S ribosomal

Table 4 Effects of methylation of ribosomes and core particles on their ability to bind ^{35}S -thiostrepton

Particles (75 pmol)	Source of crude methylase	<i>S</i> -adenosyl methionine	pmol thiostrepton bound after reconstitution (% control value)
<i>S. coel.</i> 70S	—	—	64 (100%)
<i>S. coel.</i> 70S	<i>S. az.</i>	—	63 (98%)
<i>S. coel.</i> 70S	<i>S. az.</i>	+	65 (100%)
<i>S. coel.</i> 1M cores	—	—	54 (100%)
<i>S. coel.</i> 1M cores	<i>S. az.</i>	—	43 (80%)
<i>S. coel.</i> 1M cores	<i>S. az.</i>	+	6 (11%)
<i>S. coel.</i> 1M cores	<i>S. coel.</i>	+	41 (76%)
<i>S. coel.</i> 4M cores	—	—	51 (100%)
<i>S. coel.</i> 4M cores	<i>S. az.</i>	—	38 (75%)
<i>S. coel.</i> 4M cores	<i>S. az.</i>	+	4 (8%)

Purified ribosomes or 1M core particles (derived as in Table 1) were incubated for 6 h at 30 °C with and without crude methylase in the presence and absence of 4 mM *S*-adenosyl-methionine in TMA β buffer containing 10 mM KCN (freshly prepared). Cores were then reconstituted by incubation with 1M split proteins as in Table 1. The ability of ribosomes and reconstituted cores to bind ^{35}S -thiostrepton (214 c.p.m./pmol, 70 pmol added) was assayed as in Table 1. Crude methylase preparations were obtained by centrifuging ribosomes through TMA β buffer modified to contain 1M NH_4Cl . The supernatant was dialysed against TMA β buffer and was used without further treatment.

subunits; as a consequence, thiostrepton fails to bind to such ribosomes. Because many other antibiotic-producing organisms share with *S. azureus* a similar problem of self-defence against their own toxic (antibiotic) products, it will not be surprising if they adopted similar solutions to the problem. I therefore anticipate the discovery, in organisms producing other antibiotics, of further enzymes capable of modifying ribosomal components so as to confer drug resistance on those organisms. Indeed (B. Weisblum, personal communication¹⁶), there seems to be a specific methylation of 23S ribosomal RNA in *S. erythraeus*, the actinomycete which produces erythromycin, whose ribosomes have a low affinity for erythromycin¹⁷. This organism shows a pattern of resistance (MLS phenotype) identical to that of plasmid-containing strains of *Staphylococcus aureus* which are resistant to erythromycin. In these latter strains, resistance is induced by sub-inhibitory levels of erythromycin and is accompanied by specific methylation of 23S ribosomal RNA¹⁵.

Not all antibiotic-producing organisms may defend themselves by modifying intracellular drug targets. As an alternative, the drug may be modified and thereby inactivated. Thus, various actinomycetes producing antibiotics of the aminoglycoside-aminocyclitol class possess antibiotic-inactivating enzymes similar to those present in clinical isolates of antibiotic-resistant bacteria¹⁸.

The mechanism of resistance to thiostrepton in *S. azureus* can be contrasted with that observed in spontaneously arising bacterial mutants. Such mutant strains of *B. subtilis*¹⁹ possess an altered form of ribosomal protein L11, the protein which has been implicated in the binding of thiostrepton to ribosomes of *E. coli*⁴. Clearly, therefore, ribosomes can be rendered refractory

to thiostrepton by modification of either RNA or protein components.

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Induced intragenic recombination in yeast can occur during the G₁ mitotic phase

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The conditional cell division cycle yeast mutants cdc have been used to demonstrate that intragenic recombination induced by ultraviolet or γ rays occurs in diploids arrested in G₁, a short time after irradiation and before the initiation of the S phase. This implies that pairing of homologous chromosomes does not require duplicated chromatids.

In eukaryotes, recombination can occur during both the meiotic and the mitotic cycles, although much less frequently during vegetative growth. Pairing of homologous chromosomes and exchanges between chromatids are known to occur in meiosis after the premeiotic S phase, at the four-strand stage¹⁻³. By analogy it might be thought that during mitosis recombination would occur only during the G₂ phase, and it has long been known that crossing-over can take place then^{4,5}. There is nothing inherent in recombination that requires exchanges to occur exclusively in a particular phase of the cell cycle and recombination between phages can occur in the absence of DNA replication^{6,7}. However, the possibility of pairing of chromosomes could be limited to the G₂ phase and that could restrict recombination to this stage. I report here that in the yeast *Saccharomyces cerevisiae* recombination can occur in the G₁ phase between unreplicated chromosomes.

Genetic analysis of mitotic segregants has shown that spontaneous or induced recombination may occur during the G₁ phase (refs 8 and 9, and M. Esposito, personal com-

munication). I have used a more direct approach with the thermosensitive cell division cycle (*cdc*) mutants of *S. cerevisiae* isolated by Hartwell¹⁰. When shifted during growth from the permissive to the restrictive temperature (24 and 36 °C), these cells stop dividing. For some mutants, division stops in the first cycle when the cell first reaches the sensitive step ('execution point'). The position of this step in the cycle depends on the *cdc* gene that is mutated. Heteroallelic diploids with two noncomplementing recessive mutations in a *cdc* gene can be used to show whether or not induced intragenic recombination in this *cdc* gene can occur at a given stage of the cycle: if arrested cells are irradiated and kept under the restrictive conditions, the induced recombination must occur at the blocked stage, as the *cdc*⁺ gene product is necessary for the cell to escape this block.

Induction was achieved by ultraviolet and γ -ray irradiations. The ultraviolet source was a low-pressure mercury lamp and the dose rate was 0.5 J m⁻²s⁻¹, measured by a Latarjet dosimeter. The γ -ray source was ⁶⁰Co and the dose rate 3 krad min⁻¹, determined by ferrous sulphate dosimetry. The media used were solid and liquid YEPD¹¹. Cultures were sonicated to dissociate clumps and doublets. The strain used was a *cdc4* heteroallelic diploid, of genotype *a/a cdc4-1/cdc4-3 leu1/tyr1/+ ade1/+ ade2/ade2*, obtained from H. Roman's collection. [*CDC*⁺] revertants are produced by intragenic recombination in the *cdc4* gene. Exponential growing populations of heteroallelic diploids contain 1-10 [*CDC*⁺] per 10⁶ cells. Exposure to restrictive conditions does not in itself induce

recombinants, as the following experiment shows. Heteroallelic cells were plated, kept for 0, 2, 4 or 6 h at 36 °C and incubated at 24 °C. They were then replica-plated and incubated at 36 °C. The numbers of $[CDC^+]$ colonies were the same on all the plates, showing that a 6 h exposure to 36 °C did not induce recombination.

Exponentially growing $[cdc4]$ mutants, when shifted from 24 °C to 36 °C are blocked before DNA replication. There is at least one other genetically controlled step ($cdc7$) between that of $cdc4$ and the start of DNA synthesis¹². The cells are therefore arrested in G_1 , defined with respect to DNA content. However, RNA and protein synthesis are not affected, the cells continue to grow in mass and form elongated buds. Mitochondrial DNA replication is not controlled by this gene¹³. Therefore since DNA determinations had to be made, a *petite* yeast without mitochondrial DNA (p^0) was used. It was induced on YEPD plates containing ethidium bromide ($40 \mu\text{g ml}^{-1}$).

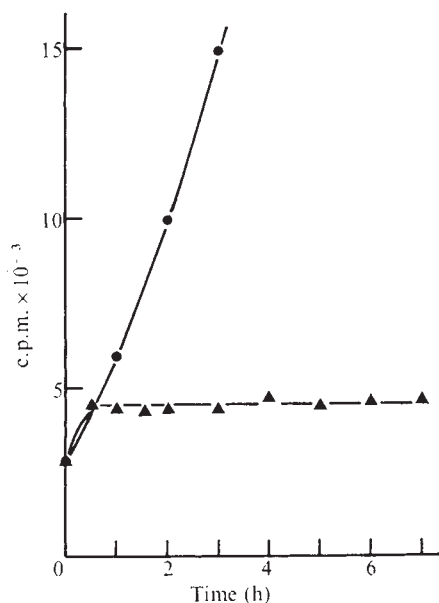


Fig. 1 Incorporation of ^{14}C -adenine in the DNA of p^0 growing cells in permissive (24 °C) (●) and restrictive (36 °C) (▲) conditions. Exponentially growing cells at 24 °C ($1-2 \times 10^7$ cells ml^{-1}) were incubated for 60 min with ^{14}C -adenine ($2.5 \mu\text{Ci ml}^{-1}$) added to the YEPD liquid medium. At the end of this period (time 0), half of the culture was transferred to 36 °C in usual growth conditions; the other half was kept at 24 °C. Constant aliquots (0.8 ml) were removed at different times and the incorporation of ^{14}C in the DNA was determined as follows: 2 ml of 2N NaOH was added to each sample. After one night at room temperature, 0.5 ml of 8% tetrasodium diphosphate was added, and DNA was precipitated by 50% trichloroacetic acid (TCA) in the cold. The samples were then filtered on GF/C filters, washed with cold 5% TCA containing 2% tetrasodium diphosphate and with 95% ethanol, and radioactivity was counted in 5 ml of a toluene-based scintillation fluid.

Figure 1 shows that incorporation into DNA was blocked completely within 1 h of the shift to 36 °C. The residual incorporation reflects DNA replication in cells that had reached a stage between the $cdc4$ execution point and the end of the S phase at time 0 of the shift. These cells, and those between the end of S and cell division, divided at 36 °C and were blocked when they reached the $cdc4$ step. Therefore 150 min after the shift (more than one generation time for $[CDC^+]$ recombinants at 36 °C) all cells were arrested in G_1 at the $cdc4$ block. Ultraviolet irradiation (12.5 J m^{-2} , as described for Fig. 4) and γ rays (2 and 4 krad) at time 0 of the shift or 150 min later did not result in any detectable incorporation of additional label into the DNA, measured as described for Fig. 1. This does not exclude repair DNA synthesis which cannot be detected in these conditions, but it indicates that after irradiation, chromosomal replication remains blocked.

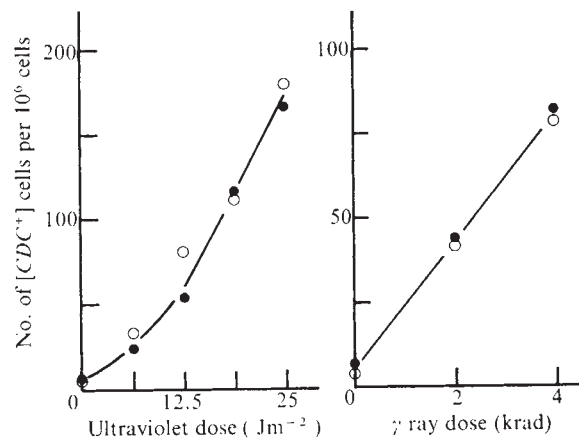
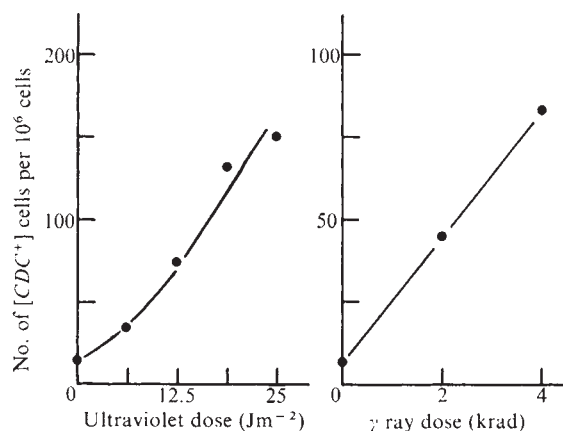


Fig. 2 Induction of $[CDC^+]$ recombinants by γ and ultraviolet irradiation of exponentially growing cells at 24 °C. Comparison of the response of the *grande* and *petite* strains. Sonicated cells ($1-2 \times 10^7 \text{ ml}^{-1}$) were irradiated with γ rays in the liquid medium and plated on warmed plates (36 °C). Ultraviolet irradiation was applied on the plates. Diluted aliquots were plated and incubated at 24 °C for counting viable cells. No lethality was induced by these doses. Double cells and cells with buds larger than one third of the mother cell were counted as two cells because their genome is duplicated. Open symbols correspond to *grande* cells; closed symbols to *petite* cells.

Because the lack of respiratory capacity in these cells could have modified the recombinational effect of radiation, I compared p^+ and p^0 cells. When exponentially growing cells in permissive conditions were irradiated with ultraviolet or γ rays, plated (on warmed plates) and incubated at 36 °C, recombination was induced with the same efficiency in both populations (Fig. 2). In none of the recombination experiments reported here was there a significant difference between the p^+ and the p^0 cells and only the results for p^0 are presented.

In the above experiment, the cell populations were at all stages of the mitotic cycle when irradiated. On the plates at 36 °C they progressed to the $cdc4$ block where they remained. If some stage favours recombination, then that event would have occurred preferentially in a subpopulation of cells at that stage when irradiated or passing through the stage before being blocked. Thus cells blocked at one stage should have an inducibility different from that of random cells. If, for instance, recombination occurs specifically during the G_2 phase, no induction of $[CDC^+]$ should be observed in $[cdc4]$ blocked diploids. In fact, this prediction does not hold. The induction

Fig. 3 Induction of $[CDC^+]$ recombinants in p^0 cells arrested in G_1 at the $cdc4$ block. Exponentially growing cells at 24 °C were blocked by a shift to 36 °C for 150 min. Cells were treated as described for Fig. 2. No correction is made in this case for budding cells because they contained a single genome.



observed in cells irradiated after 150 min of incubation at 36 °C (Fig. 3) is the same as that for cells irradiated at time 0 (Fig. 2). Irradiation with ultraviolet and γ rays induced recombinants in cells arrested in G_1 as effectively as in random cell populations.

This does not reflect a particular behaviour of [*cdc4*] cells: in similar experiments with a diploid heteroallelic in the *cdc25* gene, radiation also induced [*CDC*⁺] recombinants (data not shown). The execution point of *cdc25* is in the G_1 phase, before that of *cdc4*. Therefore, it is possible that exchanges can occur at any time during the G_1 phase. But of course recombination could also take place during the other phases of the cell cycle.

One could argue that a few cells could have escaped the *cdc4* block and that the recombination was detected in them. DNA determination in restrictive conditions (Fig. 1) shows that such cells should have constituted less than 5% of the population: incorporation of label would have been detected if 5% of the population duplicated its DNA at 36 °C. The possibility of the existence of that 5% was ruled out by experiments (to be reported elsewhere) in which the irradiated cells were allowed to divide at 24 °C before the shift to 36 °C. In those conditions there should have been at least 20 times more recombination since it could have occurred in the whole population. This was not observed.

It is important to know when the induced recombination occurred. The [*cdc4*] cells incubated at 36 °C undergo unbalanced growth and finally die (loss of colony-forming ability). If some cell death occurs before recombination, a fraction of the potential recombinants may be lost and a subpopulation could be selected. Thus, the number of viable cells (determined by incubation at 24 °C) would not correspond to the cells in which recombination may occur. Therefore I investigated the kinetics of cell inactivation and the timing of recombinational events. Growing cells were incubated for 150 min at 36 °C, so that they were blocked at the *cdc4* step. Dilute aliquots were plated on warmed plates (time 0) and incubated for different times at 36 °C before being shifted to 24 °C. Survival curves for unirradiated cells are shown in Fig. 4. Irradiation with ultraviolet (12.5 and 25 J m⁻²) and γ rays (2 and 4 krad) at time 0 did not modify the inactivation kinetics. Less than 15% of the colony-forming ability was lost during the first 3 h.

A good estimate of the timing of recombination was obtained by following the divisions of the [*CDC*⁺] cells in the liquid medium at 36 °C. Aliquots were plated at various times and kept at 36 °C (Fig. 4). In non-irradiated populations, the number of colonies increased exponentially as a function of time. It corresponded to the divisions of the [*CDC*⁺] pre-existing cells and showed that new recombinants were not induced by the restrictive conditions. In the irradiated populations, the number of pre-existing and induced recombinants was known from the platings at time 0. Any increase in this number as a function of time in liquid medium will be due to cell divisions of pre-existing or induced [*CDC*⁺] recombinants. This increase will be exponential when all the potential [*CDC*⁺] cells divide, that is when all recombinational events have taken place. These events therefore occur during the lag which precedes this exponential increase in [*CDC*⁺] cell number. It can be estimated that the first cell division of the induced [*CDC*⁺] recombinants occurs on average at the end of the lag although it may happen slightly earlier if the heteroduplex is not repaired in some recombinants: in that case the first cell division of the revertant would give rise to only one [*CDC*⁺] cell, the mother or the daughter cell remaining [*cdc*⁻]. After recombination in the *cdc4* gene, revertant cells have to pass through the stage leading to cell division. This takes at least two thirds of the cell cycle time (130 min) of [*CDC*⁺] recombinants growing at 36 °C. Recombination therefore had to occur at least 85 min before the end of the lag. From Fig. 4, I deduce that after exposure to γ rays (2 and 4 krad) recombination occurred within 95 and 115 min respectively, and

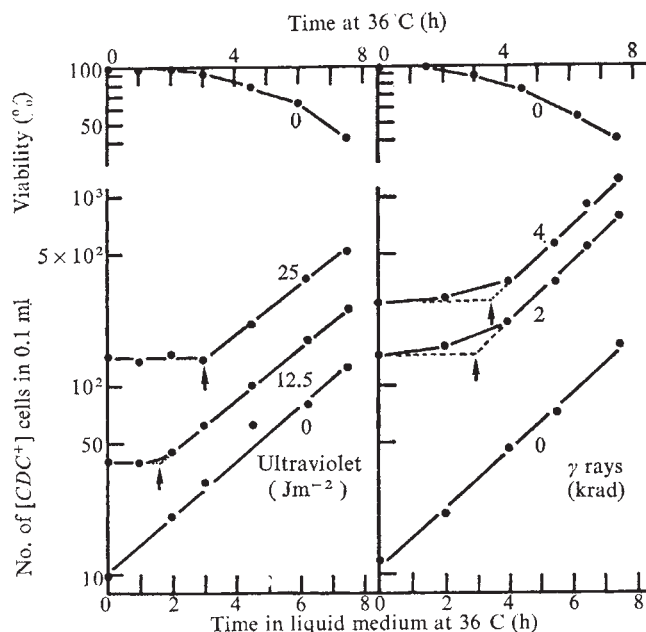


Fig. 4 Viability of [*cdc4*] heteroallelic cells and increase in the number of [*CDC*⁺] recombinants, as a function of time at 36 °C. Growing cells ($1-2 \times 10^7$ ml⁻¹) at 24 °C were arrested by 150 min of incubation at 36 °C (time 0). Viability was measured by plating dilute aliquots of blocked cells and incubating the plates for different times at 36 °C before reincubation at 24 °C. Divisions of the [*CDC*⁺] recombinants were measured by plating aliquots at 36 °C, at different times. For ultraviolet irradiation, cells were collected on Millipore filters, suspended in saline at the same cell concentration, irradiated, collected on filters and resuspended in warmed YEPD medium. Cells were irradiated with γ rays in the liquid growth medium. Arrows indicate the end of the lag period.

after ultraviolet irradiation (12.5 and 25 J m⁻²) within 20 and 95 min respectively. The ultraviolet-induced delay before cell division is known to be dose dependent, and is presumably related to the blockage of transcription by dimers¹⁴. Very close values have been obtained with random cells growing at 24 °C, irradiated and immediately transferred to liquid medium at 36 °C. Therefore, incubation for 150 min at 36 °C before irradiation did not significantly affect the timing of recombination. I conclude that recombination occurred soon after irradiation and before any significant cell inactivation.

These experiments demonstrate that induced intragenic recombination can occur during the G_1 phase in the absence of major DNA replication, and they are in agreement with the conclusions of Wildenberg⁸, Henaut⁹ and M. Esposito (personal communication) based on genetic analysis.

It has often been reported that a transient block in DNA replication¹⁵⁻¹⁹ produces recombinants that can be explained by the introduction of lesions in the DNA. This is not the case in the [*cdc4*] mutant: the block in DNA replication is an indirect consequence of the mutation and occurs before the start of the S phase. Because exposure of the cells to the restrictive conditions does not by itself induce recombination, the results reported here are not a consequence of a particular recombinational behaviour of the *cdc4* cells in my experimental conditions.

Formal genetic analyses have shown that mitotic recombination can occur during the G_2 phase^{4,5,20-22}. It is also known that in *Ustilago maydis* intragenic recombination induced in G_2 cells by γ rays²³ or by restrictive conditions in a temperature-sensitive mutant blocked for chromosome replication¹⁹, takes place during the G_2 phase. My results demonstrate that induced intragenic recombination occurs at least as frequently during the G_1 as during the G_2 phase, and that the pairing of homologous chromosomes does not require duplicated chromatids.

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Why do nucleic acids have 3'5' phosphodiester bonds?

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Details of the stereochemistry of the 2'5' and 3'5' dinucleoside monophosphates of polynucleotides have been delineated in aqueous solution using nuclear magnetic resonance spectroscopy. Incorporation of these experimentally determined geometries into the structure of polynucleotides reveals that the intrinsic spatial configurations of the 2'5' bonds cannot support helical structures whereas the geometries of 3'5' bonds allow the formation of helical configurations for RNA.

NMR STUDIES of 3'5'-ribodinucleoside and deoxyribodinucleoside monophosphates^{1–5} which form the building blocks of RNA and DNA have provided detailed information about the conformation of these molecules in aqueous solution. These studies centred on conformational pluralism and stereochemical domino effects and examined the conformation changes of mononucleotides as they become incorporated into the molecular framework of a polynucleotide. Further, these studies revealed the subtle conformational differences between the ribo- and deoxyribo-series. The sugar conformation in the deoxy-series was found to show an overwhelming preference for ²E pucker, whereas in the ribo-series the preferred sugar pucker is determined by the nature of the base and stacking interactions. The torsional variation about the C3'–O3' bond (ϕ_3') in the ribo-series is governed by an equilibrium in which ϕ_3' and sugar pucker are coupled, while in the deoxy-series no such couplings were detected. Despite these differences, the conformation about the phosphodiester bond for both the series is found to prefer g[–]g[–] domain with extensive base–base interactions (right handed stack).

In contrast, there has been no systematic study of the conformational behaviour of the synthetic 2'5' linked dinucleoside monophosphates. The available data^{6–8} on 2'5' ApA and 2'5' CpC indicate that in aqueous solution both classes of dimer may have very similar conformational properties, and that the 2'5' linkage does not bring about significant conformational deviations. Then why are 3'5' linked dimers the exclusive building blocks of RNA and DNA? The theoretical calculations of Perahia *et al.*⁹ suggest that 2'5' dimers prefer a g⁺g⁺ geometry (left handed stacks) about the phosphodiester bonds. For example, for ³E–³E sugar pucker and the phosphate geometry corresponding¹⁰ to UpA2, the probability of occurrence of 2'5' linked dimers in the left handed stacked conformation (g⁺g⁺) is about 85%. So far there is no experimental evidence that suggests that 2'5' linked dimers show any preference to exist in left handed stacked arrays.

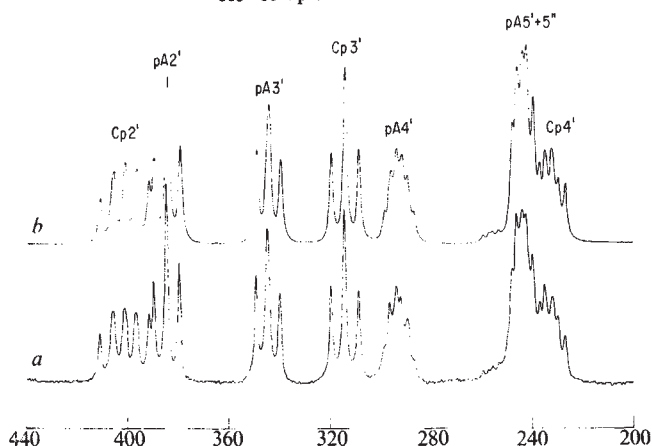
To resolve this problem, we have undertaken a systematic and comprehensive proton magnetic resonance study of the conformational properties of synthetic 2'5' linked dimers. The dinucleoside monophosphates and the corresponding monomers were obtained from Sigma. The details of sample preparation are

reported elsewhere^{1–3}. ¹H NMR spectra were recorded at 270 MHz using systems described in refs 1–3. All the spectra were analysed using the computer program LACON III. Figure 1 illustrates the observed and simulated spectra of 2'5' CpA at 20 °C. The spin-lattice relaxation times (T_1) were measured using the inversion recovery technique. Assignments of the various sugar resonances were made by homonuclear decoupling and line shape analyses. The assignments of H2 and H8 of the purine bases in the 2'5'dimers ApA, ApC and CpA were made by deuterium exchange as well as by measurement of spin lattice relaxation times. Within the set of four resonances from the bases of 2'5' ApA, the resonances having the longer relaxation time¹¹ were assigned to H2. However, the unequivocal assignment of H2 and H8 in a given nucleotidyl unit of the dimer is not possible from T_1 or deuterium exchange experiments, and this was made from the results of Mn²⁺ binding studies of Kondo *et al.*⁶. The coupling constant data derived for the various monomers and dimers were translated into conformational parameters using equations in refs 12 and 13 and the data are summarised in Table 1.

Conformational properties of ribofuranose phosphate moiety

The data indicate that in most cases (Table 1), the ribose moieties of the 5' unit of 2'5' and 3'5' dimers show a preference for ³E pucker. In addition, on dimerisation, there is an increase in the ³E conformer populations of the 5' residues. This increase ranges from a minimum of 7% to a maximum of 29%. In view of the clearly established correlation between χ and the ribose conformation^{1–4} it seems reasonable to conclude that the magnitude of

Fig. 1 The observed high field (a) and computer simulated (b) 270 MHz ¹H NMR spectra of 2'5' CpA. The chemical shifts are in Hz downfield from internal tetramethyl ammonium chloride. H5', H5'' of Cp-are not shown.



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Table 1 Conformational parameters for 2'5' and 3'5' dinucleoside monophosphates and corresponding monomers

Nucleotide		T (°C)*	2'5' Dimer				3'5' Dimer†				5' Monomer			2' Monomer			3' Monomer		
			% ³ E	%gg	%g'g'	θPH	% ³ E	%gg	%g'g'	θPH	% ³ E	%gg	%g'g'	% ³ E	%gg	θPH	% ³ E	%gg	θPH
ApA	Ap-	20	51	85	—	±31°	58	79	—	±37°	40	77	72	29	81	±31°	31	82	±36°
	-pA		61	94	85	—	61	74	90	—									
UpU	Up-	20	46	68	—	±31°	56	75	—	±35°	46	89	75	45	68	±31°	56	68	±35°
	-pU		56	84	83	—	53	83	77	—									
UpG	Up-	20	50	70	—	±31°	54	75	—	±35°	37	71	71	45	68	±31°	56	68	±35°
	-pG		59	95	86	—	50	80	81	—									
GpU	Gp-	20	31	77	—	±31°	49	80	—	±34°	46	89	75	37	72	±31°	33	72	±37°
	-pU		55	98	87	—	62	92	82	—									
CpA	Cp-	20	56	68	—	±31°	71	79	—	±35°	40	77	72	48	64	±31°	60	71	±35°
	-pA		47	92	86	—	57	90	84	—									
ApC	Ap-	20	21	93	—	±31°	64	85	—	±33°	55	77	75	29	81	±31°	31	82	±36°
	-pC		84	98	87	—	75	93	85	—									
CpC‡	Cp-	20	46	69	—	±31°	74	81	—	±35°	55	77	75	48	64	±31°	60	71	±35°
	-pC		66	86	—	—	69	92	84	—									

*Temperature

†Data taken from refs 1 and 2.

‡Data from ref. 8.

χ_2 for both types of dimer will be in the same domain. However, the 3' residues of 3'5' dimers in general show a preference for ³E conformation; no such patterns are discernible for the 2' residue of 2'5' dimers. For example, the 2' residues in ApA, UpU, UpG, CpA and CpC, display 50:50 distribution of ²E:³E conformers. In 2'5' GpU and 2'5'ApC, the ribose rings of Gp- and Ap- show a marked preference (70–80%) for ²E conformation. The differences between 2'5' and 3'5' dimers also influence the conformational changes of the ribose ring on dimerisation. In 3'5' dimers, except UpU and UpG, the dimerisation causes a shift towards ³E populations for the 3' residue and this has been interpreted as being due to stacking interactions. However, in 2'5' dimers, except for ApA, the dimerisation has very little influence on the ribose conformer equilibrium. These observations along with the known nexus^{1,2} between sugar pucker and χ strongly suggest that a major conformational difference between the 2'5' and 3'5' dimers may be associated with the magnitude of χ_1 .

The C4'–C5' and C5'–O5' bonds in both classes of dimer form a conformational network preferring the classically stable domains of gg ($\psi_1, \psi_2 \approx 60^\circ$) and g'g' ($\phi_2 \approx 180^\circ$) conformations (Table 1). However, in all the 2'5' dimers, the 5' unit shows a greater preference for gg orientation than the 2' unit. This situation is quite different from that of the normal 3'5' dinucleoside monophosphates, where for the dipurines, the 3' residue shows a greater degree of preference for gg than the 5' residue; in the diprimidines the situation is reversed.

The dimerisation does not seem to have any significant effect on the conformational preferences about the C2'–O2' and C3'–O3' bonds of 2'5' and 3'5' dimers. For the former the value is $\phi'_1 = 209^\circ/271^\circ$ and for the latter $\phi'_1 = 206^\circ/274^\circ$.

Conformation of glycosidic (χ_1, χ_2) and phosphodiester (ω', ω) bonds

In nucleic acid structures, the phosphodiester moiety (ω', ω) acts as a swivel point and changes in the magnitude of ω', ω can generate an array of conformations^{14,15} in which the molecules are stacked and destacked, and consequently there will also be changes in the magnitude of glycosidic torsion^{1–3}. There are no direct methods of determining the magnitude of glycosidic torsions ω' and ω because of the lack of appropriate nuclei for measurement of coupling constants. However, Cheng and Sarma³ have advocated a method to determine the torsion angles ω', ω, χ_1 and χ_2 based on the complete analysis of dimerisation shift data for the base and sugar protons in terms of the cylindrical coordinates ρ and z using the NMR ring current theory. The uniqueness of the solution in terms of ring current theory may not be strictly true because of the large number of variables but it will give at least correct domains for these torsion angles.

The dimerisation data for all the naturally occurring 3'5' dinucleoside monophosphates have been presented elsewhere^{1,2}. Detailed analysis of these dimerisation data for a few representative dimers (3'5' ApA, 3'5' ApC and 3'5' CpC), according to the method of Cheng and Sarma³ gives the preferred: $\chi_1 \approx 25^\circ \pm 5^\circ$, $\chi_2 \approx 50^\circ \pm 5^\circ$, $\omega' \approx 285^\circ \pm 5^\circ$ and $\omega \approx 290^\circ \pm 5^\circ$. During these calculations, the conformations about ψ_1, ψ_2, ϕ_2 and ϕ'_1 and that of the ribofuranose ring were maintained as those which have been shown experimentally to be preferred^{1,2}; that is $\psi_1 = \psi_2 = 60^\circ$, $\phi_2 = 180^\circ$, $\phi'_1 = 206^\circ$ and ³E, ³E. The conformation of 3'5' pApAp, so determined, in aqueous solution is given in Fig. 2a.

Table 2 Dimerisation shifts for 2'5' dinucleoside monophosphates

		δ (Monomer)- δ (dimer) (p.p.m.)								
Nucleotide		T(C)	1'	2'	3'	4'	5'	5''	H(2)5	H(8)6
ApA	Ap-	20	0.040	0.03	-0.081	0.077	0.092	0.129	0.303	0.344
	-pA		0.324	0.408	0.200	0.025	0.065	0.214	0.070	0.352
UpU	Up-	20	-0.042	0.063	-0.091	-0.029	0.000	0.000	0.015	-0.018
	-pU		0.098	0.094	0.084	0.126	-0.008	0.015	0.179	0.074
UpG	Up-	20	0.208	0.097	-0.080	-0.077	0.021	0.062	0.494	0.315
	-pG		-0.057	0.051	0.042	0.021	0.006	0.036	---	0.129
GpU	Gp-	20	0.000	0.105	-0.049	-0.023	0.000	0.014	---	0.048
	-pU		0.280	0.245	0.235	0.307	0.193	0.456	0.189	0.370
ApC	Ap-	20	0.000	0.057	-0.051	-0.024	0.015	0.023	0.067	0.021
	-pC		0.432	0.344	0.383	0.347	0.264	0.350	0.220	0.670
CpA	Cp-	20	0.059	0.088	0.018	0.089	0.042	0.064	0.651	0.385
	-pA		0.08	0.124	0.029	0.100	0.100	0.009	0.018	0.101
CpC*	Cp-	20	-0.014	-0.025	-0.084	0.006	-0.024	-0.018	0.235	0.131
	-pC		0.141	0.154	0.085	0.133	0.07	-0.03	0.129	0.202

*Data taken from ref. 8.

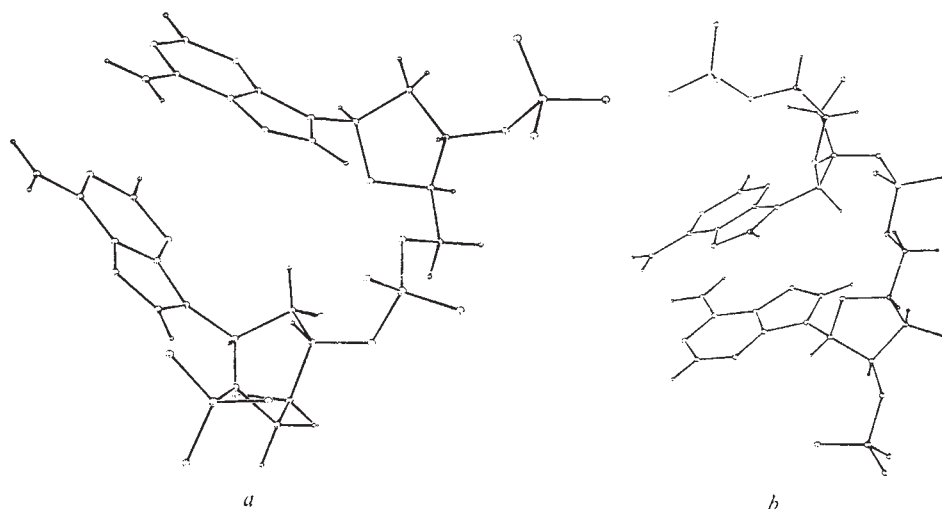


Fig. 2 *a*, The stacked spatial configuration of 3'5' pApAp in aqueous solution. The ribose of both the units are ³E; the various torsion angles are: $\chi_1 = 25^\circ$, $\psi_1 = 60^\circ$, $\phi_1' = 206^\circ$, $\phi_2 = 180^\circ$, $\omega' = 285^\circ$, $\omega = 290^\circ$, $\psi_2 = 60^\circ$, $\chi_2 = 50^\circ$. *b*, The stacked spatial configuration of 2'5' pApAp in aqueous solution; the ribose of both the units are ³E; torsion angles are: $\chi_1 = -50^\circ$, $\chi_2 = 60^\circ$, $\phi_1 = 209^\circ$, $\phi_2 = 180^\circ$, $\omega' = 295^\circ$, $\omega = 300^\circ$, $\psi_1 = 60^\circ$, $\psi_2 = 60^\circ$.

The dimerisation data for the various 2'5' dinucleoside monophosphates are presented in Table 2. Survey of the dimerisation shift data for 2'5' ApA, a purine-purine dimer, reveals that all the protons of the 5' nucleotidyl unit undergo substantial upfield shifts, the shift being largest for H2'. Comparison of the dimerisation shift data for 2'5' ApA and 2'5' CpA (in 2'5' CpA, the ribose protons of the 5' residue do not experience such large shifts as in 2'5' ApA) and comparison of 2'5' ApA and 2'5' ApC (in 2'5' ApC the ribose protons of the 5' nucleotidyl unit experience large upfield shifts) suggests that the shifts originate from the ring current fields of the purine of the 2' nucleotidyl units. This observation is also supported by a comparison of the dimerisation shift data for 2'5' UpU and 2'5' GpU. The observation of the larger dimerisation shifts for the 5' nucleotidyl units in 2'5' dimers is not in line with those observed for 3'5' ribo- and deoxyribodinucleoside monophosphates in which the protons of 3' nucleotidyl unit experience much larger upfield shifts compared to the 5' unit. This suggests either that ω' lie in a domain different from that for 3'5' dimers and/or that χ_1 and χ_2 have different values.

A search for $\omega'\omega$ was made in g^+g^+ ($\omega'/\omega = 80^\circ/80^\circ$) and g^-g^- ($\omega'/\omega = 290^\circ/290^\circ$) domains by keeping ψ_1 , ψ_2 , ϕ_2 , ϕ_1' to their experimental values, ribose in either ³E or ²E conformations and χ_1 and χ_2 constant, say zero, zero; ω' and ω were varied in steps of 20° until a combination of ω' and ω gave a reasonable base-base overlap and base interplanar distance consistent with the dimerisation data. With this set of $\omega'\omega$ values, χ_1 and χ_2 were varied until a better fit of the observed dimerisation shifts was obtained. Finally the torsion angles ω' , ω , χ_1 and χ_2 were varied by 5° to arrive at a combination which provided the best possible fit for the dimerisation shifts of protons which are expected to be affected by ring current fields, that is, the base protons, H1', H2' and H3'. It was observed that for 2'5' pApAp, the best possible combinations are $\omega' = 295^\circ \pm 5^\circ$, $\omega = 300^\circ \pm 5^\circ$, $\chi_1 = -50^\circ \pm 5^\circ$ and $\chi_2 = 60^\circ \pm 5^\circ$, and this leads to a stacked array in which the base plane

separation is about 3–4 Å, and is approximately parallel; this is shown in Fig. 2*b*. The magnitude of ρ_5 , ρ_6 and z for the protons of the 5' nucleotidyl unit in this conformation with respect to the adenine moiety of the 2' unit predicts substantial shielding of these protons. These values along with the projected ¹⁶ and observed shieldings for the 5'-unit of 2'5' ApA are given in Table 3. The data indicate a general agreement between the projected and the observed shieldings for H1', H2', H3' and the base protons. The combination of torsion angles used also reproduces the shielding effect of the adenine of the 5' unit on the protons of the 2' residue. Thus, the results indicate that a stacked molecular topology in which $\omega'\omega$ lie in the g^-g^- domain and $\chi_1 \approx -50^\circ$ and $\chi_2 \approx 60^\circ$ contributes significantly to the aqueous solution conformational blend of 2'5' ApA. It was further found that in the g^+g^+ domain the observed dimerisation trends cannot be completely accounted for. However, the observed downfield shift of H3' of the 2' residue (this downfield shift originates from factors other than ring current effects and hence cannot be included in the present calculations) is possible only in g^+g^+ orientation⁸, suggesting some minor contribution from g^+g^+ conformers to the conformational blend of 2'5' ApA.

The geometrical parameters so determined for 2'5' ApA were then used with other dimers, including 2'5' ApC, 2'5' CpA and 2'5' CpC, to determine the molecular topologies of 2'5' dimers in general. The cylindrical coordinates ρ and z calculated for these dimers based on 2'5' ApA conformation lead to a general agreement between projected and observed dimerisation shifts except for 2'5' ApC where $\omega' = 290^\circ$ and $\chi_2 = 55^\circ$ gave a better fit. Hence it is reasonable to conclude that the spatial configuration in which $\omega' \approx 295^\circ$, $\omega \approx 300^\circ$, $\chi_1 \approx -50^\circ$ and $\chi_2 \approx 60^\circ$ makes a sizable contribution to the overall conformational blend of 2'5' dinucleoside monophosphates in aqueous solution.

From the above discussion it seems that for 2'5' dinucleoside monophosphates, χ_1 resides in the low *syn* domain ($\approx -50^\circ$ or $+310^\circ$) and χ_2 in the *anti* domain ($\approx 60^\circ$). The observed χ values

Table 3 Cylindrical coordinates z , ρ_5 and ρ_6 for protons of 5' nucleotidyl unit of 2'5' and 3'5' ApA and projected and observed shielding (p.p.m.)

Protons	2'5' Dimer					3'5' Dimer				
	z	ρ_5	ρ_6	Projected shielding (p.p.m.)	Observed shielding (p.p.m.)	z	ρ_5	ρ_6	Projected shielding (p.p.m.)	Observed shielding (p.p.m.)
H1'	4.4	1.1	3.1	0.38	0.324	4.5	4.6	3.5	0.12	0.180
H2'	6.2	1.0	2.1	0.45	0.408	6.9	3.6	2.8	0.13	0.170
H3'	5.2	3.1	3.6	0.14	0.200	6.6	3.6	4.1	0.05	0.000
H4'	4.1	3.6	5.2	0.05	0.025	5.0	5.9	5.9	0.00	0.0150
H5'	2.9	4.1	5.3	0.00	0.065	3.7	5.5	6.2	0.00	-0.245
H5''	3.6	4.9	5.7	0.00	0.214	5.3	5.7	6.5	0.00	-0.051
H2	3.4	4.3	4.2	0.02	0.070	3.4	5.5	3.4	0.15	0.201
H8	4.0	2.8	2.1	0.45	0.352	5.1	1.9	2.9	0.31	0.250

The dimerisation shift is calculated using the traditionally accepted methodology involving the use of NMR to determine nucleic acid structures. The dimerisation data for the base and interior protons essentially reflect intramolecular spatial configuration. Contributions from glycosidic torsional changes are not significant because no significant changes in glycosidic torsion take place on dimerisation.

Table 4 Proton spin-lattice relaxation time (T_1) for 2'5' ApA at 20 °C pH = 8.0

Proton	2' Unit	5' Unit
H-8	1.2	1.0
H-1'	1.2	2.0
H-2	4.0	3.7
$(T_1)_8/(T_1)_{1'}$	1.0	0.5

for the 2'5' dimers are quite large compared to those reported for 3'5' dimers from X-ray data¹⁷⁻²². Hence we have attempted to obtain additional experimental confirmation for our findings. We have detailed elsewhere¹ the various experimental approaches such as pH effects, Mn^{2+} binding and NOE studies, which can be used to obtain information about χ . These approaches are qualitative, however, and cannot give the information required¹. But, it has been shown recently²³ that the *syn-anti* conformational equilibrium is manifested in the ratio of the spin-lattice relaxation time (T_1) of H8 and H1'. This ratio will be more than unity for *syn* conformation and less than unity for *anti*. The spin-lattice relaxation times for H8, H2 and H1' of both the nucleotidyl units of 2'5' ApA are given in Table 4. The data reveal that the ratio $(T_1)_8:(T_1)_{1'}$ for the 2' nucleotidyl unit is 1.0 and that for the 5' residue is 0.5. This clearly establishes that the glycosidic torsion χ_2 is in the normal *anti* range while χ_1 has a bias for *syn* conformation. This is also qualitatively supported by the following arguments. (1) The dimerisation shift data for the 5' nucleotidyl unit, especially the largest shift for H2', can be explained only when $\chi_1 \approx -50^\circ$. This might also be the reason for the observed difference in the dimerisation shift data between the 3'5' and 2'5' dimers. (2) The NOE data²⁴ on 2'-AMP suggests that the glycosidic torsion has a preference for *syn* conformation. When 2'-AMP is incorporated into the dimer it is quite likely that the glycosidic torsion becomes adjusted to a value which is closer to that in the monomer rather than flipping to the normal *anti* range. (3) Crystal and molecular structure studies on²⁵ β -adenosine-2'- β -uridine-5'-phosphoric acid give a value of -54° for χ_1 and -6° for χ_2 .

This article represents the first report of this unusual range for the glycosidic torsion angle, especially for χ_1 , and this observation

has profound implications with respect to the helical configurations of polynucleotides with 2'5' linkage.

Stereochemical properties of polynucleotides with 3'5' and 2'5' phosphodiester bonds

The conformational parameters of dinucleoside monophosphates obtained from single crystal X-ray studies are similar to those obtained for nucleic acid fibres and can be generally extrapolated to explain the helical properties of nucleic acids¹⁴. It is usually found that the backbone torsion angles restricted to certain ranges and glycosidic torsion angles in the *anti* domain facilitate the formation of helices.

The torsion angles ($\psi_1 = 60^\circ$, $\psi_2 = 60^\circ$, $\phi_2 = 180^\circ$, $\phi_1 = 209^\circ$, 3E , ${}^3E, \omega' = 295^\circ$, $\omega = 300^\circ$, $\chi_1 = -50^\circ$ and $\chi_2 = 60^\circ$) previously obtained for 2'5' ApA when used in a repeating fashion preclude the formation of a helix. However, it was found that by keeping the backbone torsion angles fixed and varying χ_1 and χ_2 , the helices could be formed in certain domains of χ_1 and χ_2 . The combination in the ranges of $\chi_1 = -50^\circ$ to -10° and $\chi_2 = 60^\circ$ to 0° does not allow the formation of helix. In contrast for 3'5' dinucleoside monophosphates we could not find a domain for χ_1 and χ_2 in the *anti* range which cannot form helices. The magnitudes of χ_1 and χ_2 used to determine the domains of χ_1 and χ_2 in 2'5' and 3'5' dinucleoside monophosphates which facilitate helix formation are summarised in Table 5.

From the above it is clear that for a given set of conformational angles, the 3'5' dimers can generate helical structures. However, in 2'5' linked dimers the intrinsic molecular stereodynamics are such that, in order to attain base-base interactions, the magnitude of χ_1 changes so much that they are no longer able to become part of helical polynucleotides. The data in Table 5 show that 2'5' dimers can indeed become part of helical structures, but the magnitudes of χ in them are such that no stabilisation of the helix is possible by base-base stacking interactions.

One of the rules of molecular biology is that polynucleotides must be present either completely or significantly in stable helical configurations, in order to participate in the fundamental processes of information storage, transfer and retrieval. From the results reported here it is reasonable to conclude that the exclusive presence of 3'5' phosphodiester bonds in nucleic acids is due to the built-in potentiality in their constitution to generate biologically functional stable helices with base-base stacking interactions. 2'5' linkages are not encountered because of their inability to produce comparable helical configurations.

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Table 5 Glycosidic torsion (χ_1 and χ_2) domains for helix formation

Conformational angles				Sense of helix	
ω'	ω	χ_1	χ_2	2'5' Dimer	3'5' Dimer
295	300	-50	60	No helix	Right handed
295	300	-50	40	No helix	Right handed
295	300	-50	20	No helix	Right handed
295	300	-50	0	No helix	Right handed
295	300	-50	-20	No helix	Right handed
295	300	-50	-40	Right handed	Right handed
295	300	-50	-60	Right handed	Right handed
295	300	-30	60	No helix	Right handed
295	300	-30	40	No helix	Right handed
295	300	-30	20	No helix	Right handed
295	300	-30	0	No helix	Right handed
295	300	-30	-20	No helix	Right handed
295	300	-30	-40	Right handed	Right handed
295	300	-30	-60	Right handed	Right handed
295	300	-10	60	No helix	Right handed
295	300	-10	40	No helix	Right handed
295	300	-10	20	No helix	Right handed
295	300	-10	0	No helix	Right handed
295	300	-10	-20	Right handed	Right handed
295	300	-10	-40	Right handed	Right handed
295	300	-10	-60	Right handed	Right handed
295	300	50	-60	Right handed	Right handed
295	300	50	-40	Right handed	Right handed
295	300	50	-20	Right handed	Right handed
295	300	50	0	Right handed	Right handed
295	300	50	20	Right handed	Right handed
295	300	50	40	Right handed	Right handed
295	300	50	60	Right handed	Right handed
295	300	30	-60	Right handed	Right handed
295	300	10	-60	Right handed	Right handed

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letters to nature

On the origin of Uranus' rings

DERMOTT and Gold¹ have proposed that the structure of the rings of Uranus²⁻⁴ may be explained by a series of three-body orbital resonances with the satellites Ariel, Titania and Oberon. This mechanism has been considered in more detail^{5,6}, and it is found that the particular resonances involving Ariel-Titania and Ariel-Oberon pairs encounter difficulties in explaining the ring system. This has led to strong arguments against the part played by the Miranda-Ariel resonances, but we show here that such orbital resonant effects should not be completely discarded.

Aksnes⁵ and Goldreich and Nicholson⁶ have shown that the predicted radial positions of the rings disagree with those determined from observations, and even if orbital resonant effects are responsible for the formation of the rings, the Miranda-Ariel resonances should be the most important. Indeed, from the three-body resonant relation

$$qn - (p+q)n_B + pn_A = 0 \quad (1)$$

where p and q are integers and n the mean motions while the subscripts A and B denote the outer and inner satellites, it is observed that the orbital positions of Millis *et al.*'s ring 5, and Elliot *et al.*'s α , γ and ϵ_2 rings correspond closely with the Miranda-Ariel three-body resonances with $q = 1$ and $p = 10, 9, 8$ and 7 , respectively⁴.

However, Aksnes⁵ and Goldreich and Nicholson⁶ argued that it is unlikely that particles in these rings are trapped by such orbital mechanisms. This is because the Miranda-Ariel resonances allow only very small displacement ($\Delta a < 0.1$ km) from the radial positions of exact resonances and the rings all have widths (Δr) > 1 km ($\Delta r \approx 1-10$ km for the α ring and $\Delta r \approx 30-100$ km for the ϵ_2 ring).

Although this is a strong argument against the possible part played by the Miranda-Ariel resonances in the formation of the Uranus rings we believe that such orbital resonant effects should not be discarded. For one thing, there is no compelling reason for orbital resonances to produce clustering of resonant particles if other dynamical effects are also involved (obvious examples are the Kirkwood gaps in the main asteroid belt). Also, while inelastic collisions among the resonant particles will tend to disrupt the orbital resonance⁶, perturbation effects (larger amplitude of the oscillation in the orbital eccentricity, e) of the resonant particles might lead to mass accumulation in the neighbouring region.

Suppose originally we have a thin disk of small particles of millimetre size instead of a series of narrow rings. Inelastic collision between these particles will reduce their e values to essentially zero. In fact, without extra perturbation effects these particles will not interact any further and a flat disk structure seems to be the terminal configuration found in computer simulation experiments⁷⁻⁹. It has also been shown, however, that if proper perturbation effect is introduced such that the average e value is always larger than certain values capable of maintaining the collisional interaction between the particles, orbital focusing of the disk particles into a narrow ring is possible⁹. Therefore, it is tempting to speculate that the orbital perturbation effects on the particles trapped into the Miranda-

Ariel resonances will help increase the e value of particles in the neighbouring orbits. As a result, there possibly will be formation of particle rings (or 'jet streams'¹⁰) at selective locations corresponding to orbital resonances.

Because of the larger particle density in these rings, the coagulation process will lead to larger particles as compared to those in other areas, which means that these ring particles will suffer less orbital decay. If there are metre-size objects formed in these rings, such a structure will be very stable against Poynting-Robertson effects and gas drag (if any). On the other hand, the orbital distances of the small particles in regions between rings will be gradually reduced. This disk population will eventually be intercepted by different rings and consequently a pile-up of the materials near the resonant positions will be established. Therefore, the three-body orbital resonances of Miranda and Ariel act mainly as a trigger for the formation of narrow rings, and not as a trapping mechanism of freely orbiting particles.

Such orbital focusing mechanisms incorporating the orbital resonance and Poynting-Robertson effects are similar to the effect proposed by Gold¹¹. But in the present work the inelastic collision process is considered to be vital to the formation of narrow rings.

If the Miranda-Ariel resonances could account for the formation of Millis *et al.*'s ring 5 and Elliot *et al.*'s α , γ and ϵ_2 rings, the immediate question would be what causes the other series of rings, that is, Millis *et al.*'s ring 4 and Elliot *et al.*'s β , δ and ϵ_1 rings. Applying equation (1) and assuming that these rings are related to the three-body resonances of Miranda and an inner satellite not yet discovered, we find that resonance with $q = 1$, $p = 7, 8, 9$ and 10 could have the observed locations if the semi-major axis of the inner satellite is $\approx 1.03 \times 10^5$ km. For these resonances to have perturbation effects similar to the Miranda-Ariel resonances the mass of this new satellite should be similar to that of Miranda.

The sharply defined boundaries of the rings may be due to the nature of the narrowness of the resonances. This is because only particles in the vicinity of the $q = 1$ and $p = 10$ resonance of the Miranda-Ariel system (say) will be coagulated into larger bodies and retained in the near-neighbourhood of the resonance. The width of the ring should be larger than that determined by the resonance⁶, however, as we expect certain 'leakage' of the particles to the inner region. But only more detailed study could tell how this would work. Another important question to be answered is about the eccentricity produced by the resonances. There is no reliable way to estimate the e values except by the numerical calculation performed by Franklin and Colombo¹² in the study of the Mimas resonant effect on the saturnian ring particles. Similar calculations are now in progress and the results will be used to construct more quantitative model of the uranian rings.

Because we are still far from understanding the structure of the Uranus' rings, it may be important to search for alternative approaches to this interesting problem. The model presented here is basically an application of the concept of jet stream formation in the early history of the Solar System¹⁰, but also offers the opportunity of observational test. Possible confirmation of the inner satellite predicted here will be of great interest in cosmogony.

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Solar-wind sputtering of the martian atmosphere

IN the sputtering process an incident particle beam loses part of its energy to recoil motion of target atoms, some of which may escape through a nearby surface. The sputtering yield, S , is defined as the number of atoms ejected per incident particle. In the Solar System, sputtering will occur whenever the solar wind, consisting mainly of 1 keV AMU^{-1} hydrogen and helium ions, strikes a material body. Many years ago, Wehner *et al.*¹ suggested that solar wind-induced sputtering of the lunar surface should be an important cause of erosion; recently, analyses² of returned lunar material have been interpreted quantitatively³ in terms of such solar-wind sputtering. Mars provides another example of the interaction of the solar wind with a planetary body. However, in contrast to the lunar surface, the martian surface is largely protected from direct solar wind bombardment by its atmosphere. The primarily CO_2 atmosphere is thin by terrestrial standards but still opaque to the solar wind. We discuss here whether solar-wind sputtering of the martian atmosphere is a mechanism leading to significant mass loss.

As the solar wind collides with particles in the outermost portion of the atmosphere, it will cause atmospheric atoms and molecules to be sputtered in a manner similar to the case of a solid target. The magnitude of the effect depends on the extent to which such collisions actually occur. Although Mars lacks a significant magnetic field, the martian ionosphere may partially deflect the solar wind^{4,5}. However, for sputtering to occur the solar wind need only reach the uppermost regions of the atmosphere (say, an altitude $\approx 200 \text{ km}$). In fact a distortion of direct solar wind flow into a flow 'around' the planet can actually increase the sputtering yield by increasing the length of the protons' trajectory in the layers most easily sputtered. Such enhancement is well known in the sputtering of solids at oblique angles of incidence. Furthermore, a substantial modification of the proton velocity profile from that in interplanetary space can be tolerated because the sputtering yield is not a strong function of energy.

Thus, we have assumed here that all the impinging solar wind is effective in sputtering. As the mechanism by which gaseous targets may be sputtered has not been previously considered, the present calculation also illustrates how one may estimate the rate of such processes in other potentially interesting environments (such as jovian moons). We shall evaluate the martian atmospheric mass loss by solar wind sputtering by analogy with models of sputtering of solid surfaces and by using empirical data for the lunar surface.

The martian atmospheric density at an altitude of 200 km differs by a factor of $\sim 10^{14}$ from that of common solids. However, the sputtering yield is not expected to be a function of target density. At lower densities the beam particle will, on average, penetrate a surface more deeply before generating a

primary recoil. On the other hand, longer mean free paths allow secondary and tertiary recoils to escape more easily. In the upper atmosphere, the critical level, h_c , where the mean free path in the horizontal direction just equals the scale height, distinguishes approximately between a lower region of diffusive motion and an upper region of ballistic motion. If the solar wind ion interacts with an atmospheric molecule too far below h_c , the lower energy recoil cannot easily escape. Too far above h_c , the probability for generating a recoil is vanishingly small. This is analogous to the case of a solid where sputtered atoms originate from within the top few monolayers⁶. Of course, sputtering results from a complex network of sub-surface collisions⁶ and this must also be true in the atmospheric example. To illustrate the scales involved for Mars, if we assume a 200 K isothermal atmosphere, we then obtain the scale $H \approx 10 \text{ km}$ and the critical level $h_c \approx 200 \text{ km}$ above the surface. The horizontal mean free path for a low energy recoil atom here is $\sim 10 \text{ km}$, which may be compared with the corresponding interatomic spacing within a typical solid of $\sim 2 \text{ \AA}$.

A further possible difference between the sputtering of a solid and an atmosphere lies in the nature of the target binding forces. To escape from a solid target, a recoil atom must have sufficient energy to overcome chemical bonds. The appropriate surface binding energy is usually taken to be the sublimation energy whose value is a few eV. In an atmosphere, the binding is provided by the gravitational field. Thus, on Mars an energy of $\sim 5.3 \text{ eV}$ is required for a CO_2 molecule to escape from an altitude of 200 km and, therefore, these chemical and gravitational energies are comparable.

We conclude from these comparisons that sputtering should proceed in much the same way in the martian atmosphere as on the lunar surface. The sputtering-induced mass loss from the Moon has been estimated to lie in the range 1 to $13 \times 10^6 \text{ atoms cm}^{-2} \text{ s}^{-1}$ which corresponds to the erosion of a surface layer of thickness 0.05 to $0.5 \text{ \AA yr}^{-1}$ (ref. 7). For this discussion, we adopt a yield of $7 \times 10^6 \text{ atoms cm}^{-2} \text{ s}^{-1}$. This implies a sputtering yield of 3×10^{-2} atoms per solar wind ion.

These yields are consistent with measured values for solids involving keV projectiles and targets of mass similar to those of the constituents of the lunar surface⁸. Furthermore, those yields are consistent with the predictions of standard theoretical models^{6,9}. (In these theoretical models of sputtering the binding of atoms in the bulk of the solid to their lattice sites is neglected. In this respect such theories are especially applicable to the treatment of gas targets.) Taking into account the fact that the solar wind flux is decreased by a factor of $(1.5)^2$ at the orbit of Mars, we estimate the mass loss from the planetary atmosphere to be $2.45 \times 10^{21} \text{ CO}_2 \text{ molecules s}^{-1}$. Integrated over the course of $4 \times 10^9 \text{ yr}$, the total mass loss becomes $3.1 \times 10^{31} \text{ CO}_2 \text{ molecules}$, a number independent of variations in the density of the atmosphere during its evolutionary history. This result may be compared with the present mass of the martian atmosphere of $4 \times 10^{31} \text{ molecules}$ (calculated on the basis of a ground level pressure of 7.5 mbar (ref. 10)).

Given the necessarily approximate nature of the present calculations, the interpretation of these results is that solar-wind sputtering must be considered as a potentially important mechanism for atmospheric mass loss from Mars. Such erosion processes will occur in much the same way in any environment where energetic particles strike a localised gas.

In conclusion, beside mass loss, sputtering may also lead to preferential loss of light elements and isotopes. This phenomenon occurs during ion bombardment of many alloys and compounds^{11,12} and has been proposed³ as the source of the isotopic fractionation observed on the surface of fine lunar dust grains subjected to solar wind bombardment. It may also occur in the martian atmosphere where certain isotopic anomalies have been reported¹³. Within a collision cascade⁶, lighter isotopes recoil on average with higher velocities¹² so that they will be preferentially lost from the top of the atmosphere. The efficiency of this process for the lightest atmospheric constituents will be enhanced by the fact that their distribution extends

to higher altitudes, so that more particles are exposed to the solar wind.

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Determination of the $L\alpha_{1,2}$ and $L\beta$ X-ray emission spectrum of iron using a photoelectron spectrometer

HIGH resolution X-ray emission spectra that arise from valence band inner shell electronic transitions contain detailed and valuable information about the electronic structure of molecular orbitals or the band structure of solids¹. The operation of the dipole selection rule ($\Delta l = \pm 1$) enables s and p character from specific atoms to be identified. Unfortunately the resolution available with commercial X-ray fluorescence spectrometers ($E/\Delta E \sim 400$) is sufficient to produce spectra of only limited value. The spectral broadening inherent in an X-ray photoelectron spectrometer is usually much less than 1 eV which suggested to us that such an instrument might be modified to produce high resolution 'X-ray spectra' by the expedient of observing the photoelectron spectrum generated when a sample is irradiated with the X-ray spectrum of interest. We report here that the only modification needed to convert a photoelectron spectrometer to yield X-ray conversion spectra is a modification of the anode. C 1s in graphite was found to be an acceptable convertor level.

The photoelectron process, $h\nu = E_k + E_i + f$, can, by measurement of the kinetic energy of the ejected electron (E_k), be used to determine the electron's ionisation energy (E_i) if the energy of irradiating photon ($h\nu$) is known or vice versa, provided that f can also be found. f will depend on the work functions of the sample and the spectrometer and also on the charge of the sample. X-ray photoelectron spectroscopy, typically using Al $K\alpha_{1,2}$ ($h\nu = 1486.6$ eV) or Mg $K\alpha_{1,2}$ ($h\nu = 1,254.0$ eV) X-ray lines as the source of photons has, in combination with X-ray emission spectroscopy, led to the accurate determination of most E_i values for most atoms². However, very little attention has been paid to the converse type of experiment in which X rays of unknown energies are allowed to eject photoelectrons, whose energies can then be measured, from a

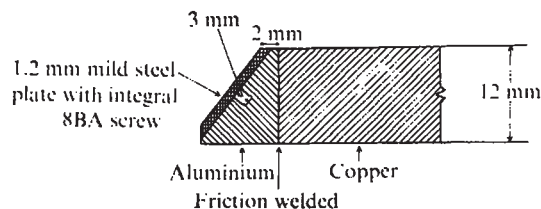


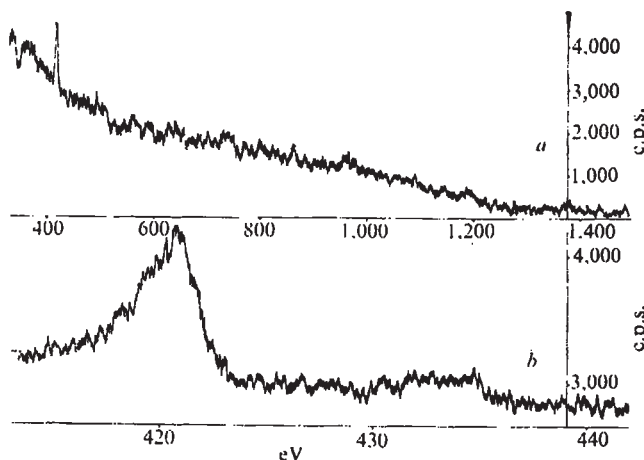
Fig. 1 Modifications to anode.

sample where the values of E_i are already known. In either the usual photoelectron experiment or the converse, it is, of course, vital that either $h\nu$ or E_i should have a very narrow energy range if the measurement of E_k is to have any value. This leads to the use of X rays as monochromatic as is possible in the photoelectron experiment and the search for materials with some narrow energy levels for the converse experiment. Clearly the narrowest levels will be found in gaseous atoms but, provided that a solid is conducting, refractory and chemically stable and has suitable levels of known E_i , it could be considered. Since the photoelectrons emitted from a particular orbital in response to irradiation by X rays will, in their energies and relative intensities, faithfully reflect the energies and intensities of the incident X rays (that is, the X-ray spectrum), the orbital level being used is called the convertor level. Hence, a suitable name for this type of spectroscopy, in which an X-ray spectrum is determined by its conversion into a corresponding photoelectron spectrum would be X-ray conversion electron spectroscopy (XCES).

Krause^{3,4} has described how a specially built spectrometer can be used to measure the X-ray emission spectra from various materials using the 1s, 2s or 2p levels of neon gas as the convertor levels, and Ebel *et al.*⁵ have demonstrated that a commercial spectrometer (McPherson ESCA-36) can be modified to obtain X-ray spectra. It is unfortunate that in this latter work gold 4f orbitals were chosen as convertors, since the doublet nature of this level ($4f_{7/2}$, $4f_{5/2}$) gave rise to two overlapping spectra corresponding to the Cu $L\alpha_{1,2}$ radiation from the copper anode.

The anode of the X-ray source used in a Vacuum Generators ESCA III, Mk.I is an aluminium block which is friction welded onto a copper rod. This rod carries the water necessary to cool the anode. The anode was modified as shown in Fig. 1 by removing a layer of aluminium about 1 mm thick from the bevelled edge and drilling a tapped hole into the aluminium. The new anode material, in this

Fig. 2 Photoelectron spectra from a graphite target using the modified anode. a, 350-1,500 V; b, 415-440 V.



case a piece of stainless steel, was then screwed into the aluminium and the edges chamfered and smoothed so that the modified anode had the same shape and dimensions as the original. It should be possible to spot-weld a steel mesh onto this new anode to receive pressed powdered samples. Also the stainless steel anode can be unscrewed and other metals or alloys (fabricated to the same shape) inserted in its place. In this way a wide variety of samples and materials might be studied by XCES.

The X rays generated by the anode normally pass through a 7.5- μm aluminium window which serves to physically isolate the X-ray source from the spectrometer, to provide a surface at earth potential, and to greatly reduce the intensity of X rays whose energy exceeds the K absorption edge of aluminium (1,559.9 eV) (ref. 2), (much high-energy bremsstrahlung is removed in this way, making the radiation from the aluminium anode more nearly 'monochromatic', thus reducing the background in photoelectron spectra).

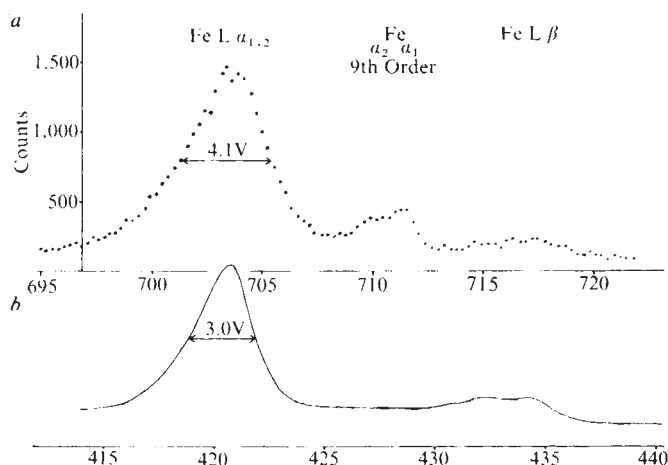


Fig. 3 *a*, X-ray emission spectrum from an iron disk using rubidium acid phthalate as the dispersing crystal. Each point represents data collected over 50 s. *b*, Photoelectron spectrum from Fig. 2*b*, smoothed by eye and corrected for rising background at about 420 V. The difference in energy between the scales in (*a*) and (*b*) is the experimental carbon 1s ionisation energy, in graphite, 703.8–420.8 = 283.0 (Fe $L\alpha_{1,2}$ peak). This differs from the literature value⁶ (284.3) by the work function of the sample relative to the spectrometer.

Results of experiments both with and without the window in place are described below.

The photoelectron spectra shown in Fig. 2*a* and *b* resulted from the irradiation of a graphite sample with iron X rays. The X-ray anode was modified as described above with a stainless steel insert and was operated at about 100 W power (10.5 kV, 10 mA). The emitted X rays passed through the aluminium window before falling on the sample. The operating pressure in both the X-ray tube and the electron analyser was about 10^{-7} τ .

The principal photoelectron emission was observed at 420.8 V; this corresponds to the ejection of photoelectrons from the 1s orbital of carbon graphite (284.3) (ref. 6) by $L\alpha_{1,2}$ X rays from iron (705 eV) (ref. 2), that is 705–284.3 = 420.7 eV. A more detailed examination of the spectrum (Fig. 2*b*) in the range 413–440 eV clearly shows photoelectrons corresponding to both $L\alpha_{1,2}$ and with much less intensity $L\beta$, X rays. The profile of $L\alpha_{1,2}$ is slightly distorted, as photoelectron peaks usually are, by an increase in the background due to the scattering of photoelectrons as the kinetic energy increases.

It is particularly interesting to compare this spectrum with the L spectrum of iron obtained using a conventional

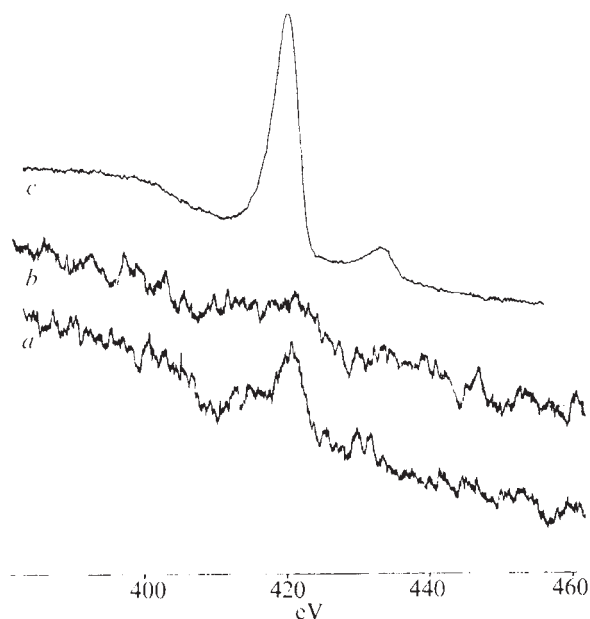


Fig. 4 Photoelectron spectra from a graphite target using the modified anode under a variety of experimental conditions, in all cases the vertical scale is arbitrary. *a*, X-ray source operated at 10 kV 5 mA. *b*, X-ray source operated at 5 kV 10 mA. *c*, X-ray source operated without aluminium window between target and anode. It was not possible to calibrate the vertical scale due to a very large zero offset. Ratemeter full scale deflection was 10^5 c.p.s.

X-ray fluorescence spectrometer (Philips PW 1410, tungsten X-ray excitation 40 mA, 60 kW) in which the X rays are dispersed using a single flat rubidium acid phthalate crystal ($2d=26.12$ Å). Such a comparison is shown in Fig. 3 where it can be seen that the XCES result, without any refinement, is somewhat superior to that from ordinary X-ray spectroscopy. The XCES data are a fold of the converter orbital natural line profile, the true X-ray emission spectrum and the spectrometer broadening function. Since the first and third can be determined it should be possible by Fourier transform techniques^{7,8} (or in other ways)^{9,10} to remove these factors from the experimental spectrum and thus reveal the X-ray spectrum alone. (It has been assumed that the X-ray spectrum would be attenuated by passage through the window but not altered, clearly this assumption could not be true over a wide range of energies and especially if an absorption edge of the window were included.)

The ability to excite X-ray emission by electrons of up to a known energy enables threshold excitation experiments to be carried out. Figure 4 shows the results obtained when the modified X-ray tube was operated at the same power of 50 W but at different voltages 5 kV, 10 mA and 10 kV, 5 mA. Only in the latter case were photoelectrons that arise from Fe $L\alpha_{1,2}$, $L\beta$ X rays observed. As the ionisation energy for Fe 1s electrons is 7,117 eV, this result clearly shows that very few Fe 2p vacancies are made by the direct ejection of 2p electrons when iron is bombarded with electrons of a few thousand volts energy. The 2p vacancies are made as a result of 2p→1s electronic relaxation to the Fe 1s vacancies which are only formed by bombardment at the higher voltage.

A final experiment was carried out to determine the role played by the aluminium window. The top trace in Fig. 4 shows that photoelectron signals can easily be observed even in the absence of the window although the background level is extremely high, as anticipated.

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Field energies and principles of equivalence

THE radial free fall motion of a charged body in a gravitational field is calculated here. A simple approach is presented, which includes the effects of the electrostatic and gravitational field energies of the falling body. The result shows that the acceleration is dependent on both mass and charge of the falling body and that both gravitational and electrostatic field energies gravitate repulsively, indicating that bodies of larger mass or larger charge will fall more slowly than bodies of lesser mass or smaller charge. Thus Einstein's principle of equivalence is not valid, as it requires that all physical systems experience the same acceleration of gravity. The approach also shows the difference between inertial and gravitational mass in a gravitational field.

The principle of equivalence denotes three related but conceptually different principles, which have been introduced by Galileo, Newton and Einstein¹. Galileo's principle maintains that point-like bodies fall in a gravitational field with the same acceleration. Newton recognised the difference between inertial, m_i , and gravitational mass, m_G . Free fall motion in Newtonian physics gives the acceleration:

$$a = (m_G/m_i)g_N \quad (1)$$

where g_N is the acceleration due to gravity. Galileo's principle implies that m_G/m_i must be the same for all bodies. By definition the standard body has the value $m_G/m_i = 1$ hence $m_G = m_i$ (Newton's principle of equivalence). Einstein extended the principle to the general statement that a homogeneous gravitational field and an accelerated coordinate system must be considered physically equivalent and therefore indistinguishable, because in both circumstances objects seem to fall with the same acceleration.

Possible violations of Einstein's principle of equivalence by the electromagnetic field of a charged body are caused by: (1) electromagnetic radiation—if the emission of radiation is an absolute phenomenon, then a homogeneous field and an accelerated reference frame are distinguishable because in the first instance radiation is observed, whereas in the second it is not, as the acceleration is only apparent. (2) Radiative reaction force—radiation carries away energy from the charged body, causing it to fall more slowly than a neutral body would in a gravitational field, whereas in an accelerated frame the rates are equal. In the special case of a homogeneous gravitational field this force is zero, despite the emission of radiation². (3) Electrostatic field energy—according to Einstein's theory of gravitation, not only mass but also energy gravitates. Therefore, the electrostatic field energy associated with a charged body will affect the body's motion in a gravitational field.

All three factors have been studied before²⁻⁶. We have used a simple approach for calculating the influence of a charged body's electrostatic and gravitational field energies on the body's motion in a gravitational field, to clarify the relationship between these field energies and the principles of equivalence.

In the Lorentz-Dirac equation of classical electrodynamics, which is often used in the discussion of Einstein's principle of equivalence^{2,4}, the effects of the electromagnetic field of a charged body on the motion of the body only occur in connection with the emission of radiation. This is because the electrostatic field does not act as a source of electrical force. Therefore, the Lorentz-Dirac equation contains no force term arising from the electrostatic field energy. In Einstein's theory of gravitation, however, both the gravitational and the electrostatic field energy gravitate and therefore produce an additional gravitational force on the body. This force term is included in the equation of motion of a charged particle in the gravitational field derived by DeWitt and Brehme⁷ and improved by Hobbs⁸, but the term seems to be closely connected to radiation from the charge and has even been denoted as the conservative component of the radiative damping force⁹. In the following we show that the gravitational effect of the electrostatic field energy is not connected with the emission of radiation.

To demonstrate the relation between field energy and the principles of equivalence, we calculate the radial free fall motion of an electrically charged point-like body in the gravitational field of a much more massive neutral body. For convenience, we consider this motion in the one-body problem as a particular case of the two-body problem.

Bazanski¹⁰ derived the equation of motion of charged particles from Einstein's field equations, as pointed out by Khan and O'Connell¹¹. With masses, charges, coordinates and velocities denoted by m_i , e_i , r_i and v_i , respectively ($i = 1, 2$), the Lagrangian for the two-body problem to order $1/c^2$ is:

$$L = \frac{m_1 v_1^2}{2} + \frac{m_2 v_2^2}{2} + \frac{m_1 v_1^4 + m_2 v_2^4}{8c^2} + \quad (2)$$

$$+ \frac{Gm_1 m_2}{r} - \frac{Gm_1 m_2}{2rc^2} [v_1 \cdot v_2 + (v_1 \cdot n)(v_2 \cdot n) - 3(v_1 - v_2)^2] - \frac{G^2 m_1 m_2 (m_1 + m_2)}{2r^2 c^2} \quad (3)$$

$$- \frac{e_1 e_2}{r} + \frac{e_1 e_2}{2rc^2} [v_1 \cdot v_2 + (v_1 \cdot n)(v_2 \cdot n)] + \quad (4)$$

$$+ \frac{G}{2r^2 c^2} [2e_1 e_2 (m_1 + m_2) - (e_1^2 m_2 + e_2^2 m_1)] \quad (5)$$

where G is the gravitational constant, c the velocity of light and n the unit vector along $r_1 - r_2$. Equations (2)+(3) is the Einstein-Infeld-Hoffman Lagrangian¹², equations (2)+(4) the Darwin Lagrangian of classical electrodynamics¹³ and equation (5), the Bazanski's term. The emission of electromagnetic radiation¹⁴ does not occur until order $1/c^3$, and that of gravitational radiation¹⁴ until $1/c^5$. Therefore, radiation effects have no role in this Lagrangian.

From the above Lagrangian we can consider the one-body problem by letting $M = m_1 \gg m_2 = m$ and $v_1 = 0$. Furthermore, if we consider only radial motion, $v_1 = \dot{r}$ and an electrically neutral central body, $e_1 = 0$, setting $e_2 = e$, we have then for the Lagrangian:

$$L = \frac{m \dot{r}^2}{2} + \frac{m \dot{r}^4}{8c^2} + \frac{GMm}{r} + \frac{3GMm \dot{r}^2}{2rc^2} - \frac{G^2 M^2 m}{2r^2 c^2} - \frac{G^2 M m^2}{2r^2 c^2} - \frac{G M e^2}{2r^2 c^2} \quad (6)$$

The potential of the last term is: $\phi = Ge^2/2r^2 c^2$. The Poisson equation, $\nabla^2 \phi = 4\pi G \rho$, yields its mass density, $\rho = e^2/4\pi c^2 r^4$. It is just the mass density equivalent ($m = E/c^2$) to the electrostatic field energy density of a spherical body, if we exclude a factor of $1/2$, which reflects the well known difference between the gravitational interaction of field energy and mass. The last term as well as the two previous terms, therefore, represent the gravitational

potential of the electrostatic field energy and the gravitational potential of the classical gravitational field energy of M and m , respectively. Although in the one-body problem the potential of the gravitational field energy of m is always small and can be neglected, we retain it here in order to clarify its influence.

Lagrange's equation yields the acceleration of gravity, $g_E = \ddot{r}$, in terms of its Newtonian value, $g_N = -GM/r^2$:

$$g_E = g_N \left(1 - \frac{3\dot{r}^2}{2c^2} - \frac{GM}{rc^2} - \frac{Gm}{rc^2} - \frac{e^2}{mc^2 r} \right) / \left(1 + \frac{3\dot{r}^2}{2c^2} + \frac{3GM}{rc^2} \right) \quad (7)$$

If the field energies of m are neglected, equation (7) shows that Galileo's principle would be valid, that is, all bodies of the same initial velocity fall with the same acceleration. Inclusion of the field energies of m , however, means that the acceleration due to gravity depends on both the mass and charge of the falling body, although it is independent of the sign of the charge. Moreover, all field energies gravitate repulsively, that is, they behave like anti-mass or negative mass. Therefore, bodies of larger mass or greater charge will fall more slowly than bodies of lesser mass or smaller charge. Thus, Einstein's principle of equivalence is not valid, as it requires that all physical systems experience the same acceleration due to gravity.

Due to the inverse mass dependence of the electrical term in equation (7), the elementary particle with the smallest mass (electron) will show the largest deviation from the Newtonian value. For an electron at the surface of the Earth $e^2/mc^2 r = 4.4 \times 10^{-22}$, which is the classical electron radius divided by the Earth's radius. It is extremely small, however, as the electrical term depends quadratically on e ; bodies of sufficiently small mass and large charge will produce a larger deviation.

We now consider the concept of mass as reflected in equation (7). Comparison of equations (1) and (7) leads to the following expressions for the inertial and gravitational mass:

$$m_1 = \left(1 + \frac{3\dot{r}^2}{2c^2} + \frac{3GM}{rc^2} \right) m \quad (8)$$

$$m_G = \left(1 - \frac{3\dot{r}^2}{2c^2} - \frac{GM}{rc^2} - \frac{Gm}{rc^2} - \frac{e^2}{mc^2 r} \right) m \quad (9)$$

where m is the rest mass and $m = m_1 = m_G$ as $r \rightarrow \infty$ with $\dot{r} = 0$. In general m_1 and m_G are not equal, therefore, Newton's principle of equivalence is violated. However, this violation is not at variance with the Eötvös-type experiments¹⁵⁻¹⁷ performed so far, which involve bodies having no radial motion ($\dot{r} = 0$) and no charge ($e = 0$), and where the gravitational field energy of m can be neglected. This leaves:

$$m_1 = \left(1 + \frac{3GM}{rc^2} \right) m \quad (10)$$

$$m_G = \left(1 - \frac{GM}{rc^2} \right) m \quad (11)$$

This absolute difference is the values between m_1 and m_G cannot be detected in such experiments because they only determine the equality of mass ratios, $m_G/m_1 = m'_G/m'_1$, of two bodies.

Following Einstein¹⁸, equation (10) is generally interpreted as the increase of inertial mass in a gravitational field. Hence, we should interpret equation (11) as the variation of gravitational mass in a gravitational field. Finally, we should point out that according to equation (9) the contribution of the 'electromagnetic mass' to the gravitational mass is given by: $m_E = -e^2/rc^2$. It is independent of the radius of the charged body and is therefore not divergent for a particle.

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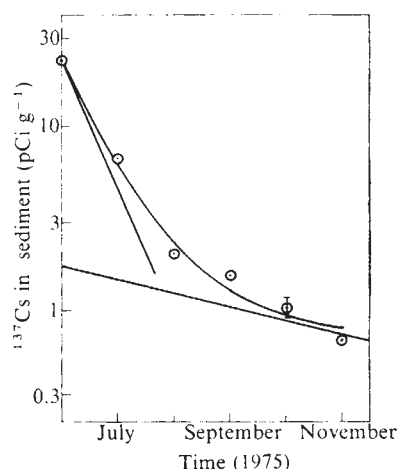
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Radionuclide loss from marine sediment

HIGH resolution γ -ray spectroscopy was used to study the concentration of radionuclides in marine sediment in Montsweag Bay, central coastal Maine, USA (the site of the Maine Yankee Nuclear Power Reactor) as a function of residence time. Loss of γ -ray emitting radionuclides with time is discussed in terms of a simple exponential description and in terms of a mechanistic model based on diffusion. Decay constants and diffusion constants for several reactor-associated γ -ray emitting radionuclides are presented.

Radionuclide concentrations in marine sediments were measured for the man-made radionuclides ^{58}Co , ^{60}Co , ^{54}Mn , ^{134}Cs and ^{137}Cs using a Ge(Li) detector with a massive lead shield and multichannel analyser. Sediment samples were collected monthly to a depth of 5 mm over sufficient area to yield 1 litre wet samples at each of four sites near the outflow of the reactor. Each sample was γ -ray counted in a standard geometry for 5,000 s. The resulting spectra were recorded on magnetic tape and computer processed using the Compton continuum subtraction method¹. The number of counts determined is converted from c.p.m. into disintegrations per second (d.p.s.) using the efficiency against energy determination for the same geometry. Branching ratios and the variation of efficiency with energy are taken into account. From d.p.s., the number of pCi g^{-1} is determined for each of the γ -ray peaks which exceed a statistical criterion; pCi g^{-1} of these radionuclides in our library of branching ratios are computed automatically and new or unidentified peaks are processed by hand calculations. The efficiency to energy curve is determined by using standard liquid sources from the Environmental Protection Agency Analytic Quality Control Laboratory, Las Vegas, Nevada in the 1-litre cylindrical fixed geometry used for all measurements. The peaks from the standard sources are analysed in the same way as the

Fig. 1 Measured ^{137}Cs concentration in marine sediment at the outflow site. A typical 2σ error bar is shown. The line through the data points, a least-squares fit to equation (1), yields values for A, B, α and β listed without parentheses in Table 1. The two straight lines correspond to the two exponential decay terms in equation (1); the upper straight line being the first term (involving A and α) and the lower straight line being the second term (involving B and β).



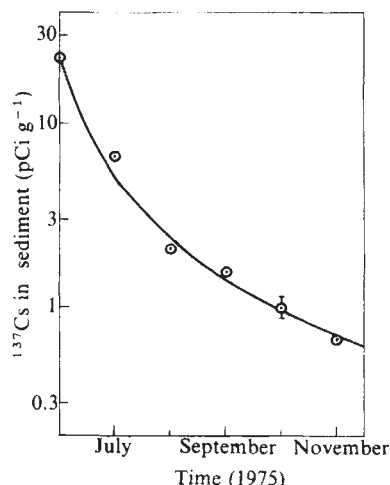


Fig. 2 Measure ^{137}Cs concentration in marine sediment at the reactor outflow site. A typical 2σ error bar is shown. The line through the data points, a nonlinear least squares fit to equations (2) and (3) yields values for the diffusion coefficient for ^{137}Cs listed in Table 1.

peaks from the unknowns. Graphical analysis of the efficiency to energy is plotted on log-log paper and tested for linearity, and consistency. Results of these periodic calibrations are used to update the computer program. New branching ratios are added to the computer program as the build-up of new radionuclides increases above the minimum detectable level.

During this three year study a 10 times larger than normal release of liquid effluents occurred (June 1975). After this release the plant changed from a surface discharge system to a subsurface diffuser system 500 m further down the bay. These two circumstances resulted in a single pulse of radionuclide effluent, providing an ideal situation in which to study the loss process for radionuclides from sediment².

Several studies of radionuclides in marine sediments have recently been discussed in terms of exponential increase and loss rates, for example, see Duursma and Gross³, and Lerman⁴. Extending the techniques discussed in these studies it is obvious that a single exponential will not describe the loss of radionuclides from sediments as shown in Fig. 1 for the typical case of ^{137}Cs at the reactor outflow. The line through the data points in Fig. 1 is calculated based on a two exponential loss process of the form of equation (1) below

$$y(t) = A \exp(-\alpha t) + B \exp(-\beta t) \quad (1)$$

where y is the concentration of radionuclide in the sediment (pCi g^{-1}), t is time measured from release, α and β are decay

rates and A and B are initial component concentrations. We have determined α , β , A and B using the constraint that $y(0) = A + B$. Two sets of values are listed in Table 1. The upper set was evaluated by nonlinear least squares curve fitting the data and then reducing the resulting rate constants (α and β) by the radioactive decay constant, λ . The lower set (in parentheses) was evaluated with the effect of radioactive decay removed from each data point before nonlinear least-squares curve fitting^{5,6}. These techniques produce rate constants (α and β) which are characteristic of loss of radionuclides from the sediment by means other than nuclear decay. The expected physical and chemical mechanisms which determine the radionuclide loss observed are diffusion, bioturbation, sedimentation and bulk transport. However, one cannot easily associate the terms in the above equation with these mechanisms either by their time constants or by their initial component concentrations. That is, although in practice, one can calculate values for these constants, it is difficult to interpret them.

The solid line in Fig. 2 is based on a theory by Lerman⁷ which includes three loss mechanisms; radioactive decay, diffusion and sedimentation. Each of these mechanisms have corresponding measurable parameters. The solutions of Lerman's theory for a single pulse from t_0 to t'_0 are:

$$C = C_0 [f(t = t_0) - f(t = t'_0)] \exp [Uz/2D] \quad (2)$$

and

$$f(t) = \frac{1}{2} \left\{ \exp \left[-z \left(\frac{U^2}{4D^2} + \frac{\lambda}{D} \right)^{\frac{1}{2}} \right] \operatorname{erfc} \left[\frac{z}{2\sqrt{Dt}} - \left(\frac{U^2}{4D} + \lambda \right)^{\frac{1}{2}} t \right]^{\frac{1}{2}} \right. \\ \left. + \exp \left[z \left(\frac{U^2}{4D^2} + \frac{\lambda}{D} \right)^{\frac{1}{2}} \right] \operatorname{erfc} \left[\frac{z}{2\sqrt{Dt}} + \left(\frac{U^2}{4D} + \lambda \right)^{\frac{1}{2}} t \right]^{\frac{1}{2}} \right\} \quad (3)$$

where U is the sedimentation rate, z is the depth parameter, D is the diffusion coefficient, λ is the nuclear decay constant, and C_0 is the concentration of radionuclides in interstitial water. The concentration C_0 is related to the concentration in solid sediment, C_b , by the equilibrium exchange constant, K , with the constraint $C_b = (K+1)C_0$.

To apply this theory, it was sufficient to use measured sedimentation rates for the estuary in question, the known decay rates corresponding to the half lives for the radionuclides observed and to let the theory predict the diffusion coefficients using the above solutions. Predicted diffusion coefficients for the five radionuclides involved in this study are listed in the last column in Table 1.

After working with both models, we conclude that although the two decay rates model can be used to fit the data, it is difficult to draw conclusions as we cannot relate parameters A , B , α and β to specific processes in the estuary. On the other hand, Lerman's theory is based on well understood mechanisms

Table 1 Theoretical parameters determined from measured radionuclide loss in marine sediment

Radio-nuclide	α ($\times 10^{-6}\text{s}^{-1}$)	β ($\times 10^{-6}\text{s}^{-1}$)	A (pCi g^{-1})	B (pCi g^{-1})	λ ($\times 10^{-6}\text{s}^{-1}$)	U (cm yr^{-1})	D ($\times 10^{-6}\text{cm}^2\text{s}^{-1}$)
^{137}Cs	0.529 (0.541)	0.0380 (0.0645)	21.3 (20.5)	1.31 (1.78)	1.03×10^{-3}	2.0	0.0130
^{134}Cs	0.518 (0.526)	0.0881 (0.0820)	9.80 (8.90)	0.689 (0.890)	1.51×10^{-2}	2.0	0.0112
^{60}Co	1.22 (1.05)	0.125 (0.136)	1.36 (1.24)	0.874 (0.844)	5.89×10^{-3}	2.0	0.055
^{58}Co	0.857 (0.906)	0.0155 (0.0104)	3.33 (2.92)	0.576 (1.000)	1.58×10^{-1}	2.0	0.067
^{54}Mn	0.865 (0.741)	0.044 (0.072)	0.587 (0.483)	0.139 (0.237)	3.72×10^{-3}	2.0	0.037

with a single parameter for each, making data interpretation straightforward. As an internal consistency test, we note from Table 1 that even though the nuclear half lives for ^{134}Cs , ^{137}Cs are quite different, the diffusion coefficients calculated from the Lerman theory agree within experimental error. The same is also true for ^{58}Co and ^{60}Co . This agreement would be expected for isotope pairs in general as their chemistry is identical and their masses are essentially the same.

Estuaries with different radionuclides, different diffusion coefficients, different sediment rates, seasonal changes in sedimentation rate, and seasonal variation of the diffusion coefficients, can be accommodated by the Lerman theory. By combining appropriate field studies with this model, we can predict the time evolution of the loss of radionuclides for a wide variety of marine environments. This predictive capability may be applied to reactor safety analysis and storage of nuclear wastes in the marine environment as well as fallout dispersion⁷.

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Termination of last interglacial episode and the Wilson Antarctic surge hypothesis

WILSON's theory¹ that Pleistocene glaciations might be initiated by massive surges of the East Antarctic ice sheet has been supported on climatological grounds by Flohn², and Hollin³ outlines several geological tests of the idea. If a surge occurred, sea level would rise rapidly by many metres and, according to Hollin and Flohn, the oceans would cool significantly—especially the Atlantic. The sea-level rise would quickly be followed by falling sea level as northern continental ice sheets developed. Numerical values approximately would be (1) sea-level rise of 10–20 m over a surge period of several hundred years³; (2) global cooling of about 1° by virtue of the albedo of the greatly increased floating ice-field in sub-Antarctic waters produced by the surge², and (3) the high sea level should persist for no more than a few hundred years as northern continental glaciation is envisaged as starting to form when the peak is reached, if not before, and this ice volume is likely to equal many metres of sea-level equivalent after 1,000 yr (refs 2,3). An alternative surge hypothesis for glacial initiation by Mercer⁴ invokes rapid disintegration of the West Antarctic ice sheet—believed to be inherently unstable because its base is extensively below sea level⁵. Rapid disintegration would cause a sea-level rise of about 5 m, perhaps as rapid but smaller in magnitude than Wilson's East Antarctic surge, followed about 1,000 yr later by mild warming (0.5–1 °C) of equatorial Atlantic temperatures as production ceased of Weddell Sea bottom water⁶. The time lag between West Antarctic ice disintegration and northern glacial initiation is uncertain

under this hypothesis, but may be a few thousand years if circulation changes of the deep Atlantic ocean are involved. Evidence presented here of sea-level changes and temperature variation argues against the Wilson hypothesis but does not preclude all 'surge' mechanisms.

These theories can best be tested geologically for the last inter-glacial maximum episode, from about 135 to 120 kyr ago as this is better documented than earlier interglacial phases. Sea-level variations are best known from coasts where coral reefs are extensively dated by $\text{Th}^{230}/\text{U}^{234}$, especially Barbados⁷, New Guinea^{8,9}, Timor¹⁰ and Hawaii¹¹, where studies show that a major sea-level rise terminated with sea level near the present level of 130–135 kyr ago, and that sea level started to fall rapidly at 118–120 kyr. Shackleton and Matthews¹² show that this interval correlates with the last interglacial as recorded in deep sea cores. From New Guinea and Timor it is established that sea level fell by several metres, relative to these islands, and then rose again sometime between 130 and 120 kyr ago^{8,10}. These are tectonically rising islands and it cannot be established whether this post-130 kyr minor fall was an absolute movement of sea level, although the minor rise seems to have been an absolute movement¹⁰. Evidence from Hawaii¹¹ suggests that both the minor fall and rise were absolute movements. Sea level interpretations from these localities are summarised in Fig. 1a. Curve A is consistent with the surge-glaciation hypothesis.

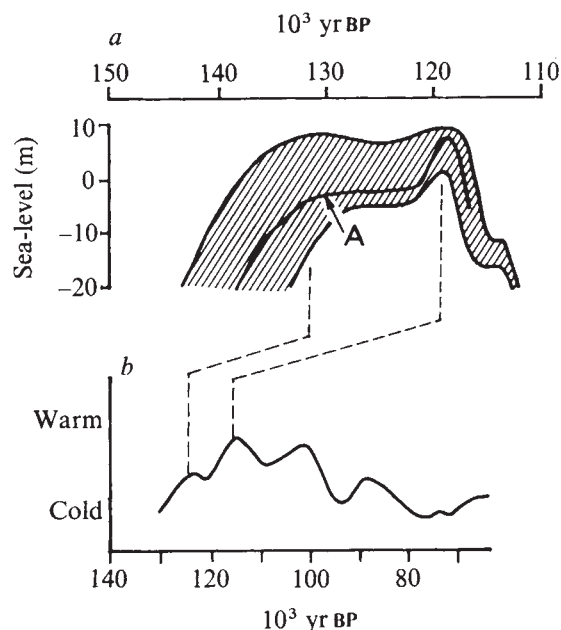


Fig. 1 a, Envelope of sea-level change, 140–110 kyr ago, from dated raised coral reefs in Papua New Guinea and Timor^{8–10}. b, Correlated temperature changes identified in Gulf of Mexico cores¹⁰.

Testing of curve A must show (1) that the rise to the sea level peak at about 120 kyr is rapid, and (2) that the peak is short-lived. Evidence at Timor and New Guinea does not deny the first test point. The bulk of the relevant reef complexes were built during the main transgression and its culmination at 130–135 kyr (refs 8, 10). In both cases the 120 kyr reef abruptly overlies an erosion surface, sometimes capped by regressional gravels, developed on the 130 kyr reef¹⁰. The same seems to be true at Hawaii¹¹. In Timor¹⁰ and Hawaii¹¹ the reef formations of the second (120 kyr) phase are small (less than 2 m thick), suggesting a short sea-level peak, which favours point (2) for curve A, although in Timor the original extent of this reef has been reduced by cliffing¹⁰. In New Guinea, however, the reef of this phase (New Guinea reef VIIb) is seen to be built of shallow water corals for a thickness

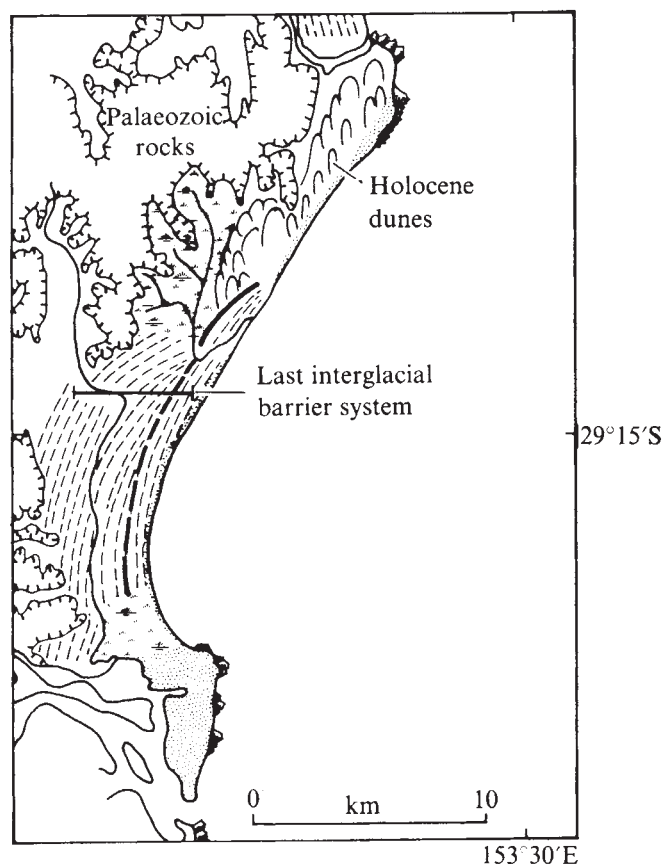


Fig. 2 Beach ridge barrier system built 140–120 kyr ago in northern New South Wales, Australia. Dashed lines show individual ridges; heavy dashed line traces plan discontinuity.

of at least 8 m and probably more (J. C., personal observations on reef VIIb in Bwangam River gorge, 1 km north-west of Sialum type section, see refs 8, 9). By analogy with Holocene reef sections in the New Guinea sequence, closely dated by ^{14}C , 8 m vertical growth of shallow water reef represents about 2,000 yr (ref. 13). This argues against the very short peak implied by the surge hypothesis.

On the northern coast of New South Wales, Australia, broad bay-barriers composed of multiple beach ridges have been dated by $\text{Th}^{230}/\text{U}^{234}$ between ~ 120 and 140 kyr (ref. 14). Isolated hermatypic corals from a transgressive facies near Newcastle ($150^\circ 49' 00'' \text{E}$, $32^\circ 45' 12'' \text{S}$) indicate that sea level was rising towards a higher-than-present level 140 kyr. At Evans Head ($153^\circ 23' 30'' \text{E}$, $29^\circ 06' 10'' \text{S}$), hermatypic corals in growth position, just above present LWM, occur within a muddy sand facies behind a Pleistocene barrier (Fig. 2). These are dated close to 120 kyr (ref. 14), and are capped by 4 m of lagoonal clay representing sedimentation at a sea level higher than present. A disjunction in beach-ridge trends on this 120–140 kyr barrier system suggests an hiatus in deposition (Fig. 2). On either side of this plan-view discontinuity the beach ridges are 8–10 m above present sea level. We believe that the outer beach-ridge set in Fig. 2 cannot be related to a high sea level younger than 18–120 kyr because the maximal sea level relative to the New South Wales coast represented by both beach ridge sets and associated lagoonal facies is $5 \pm 1 \text{ m}$ above present sea level, whereas the best available evidence indicates that 'interstadial' sea levels younger than 118 kyr were at least 8 m lower than the 118–135 kyr levels^{7,10}.

By analogy with the well-studied Holocene beach ridge systems of New South Wales¹⁵ two conclusions emerge. First the Holocene systems show no plan discontinuities like that shown in Fig. 2. Eustatic sea-level oscillations of greater than

$\pm 1 \text{ m}$ are unknown in the Holocene sequences of New South Wales¹⁵. The hiatus between the Pleistocene ridge sets, therefore, may best be explained by a fall in sea level of more than 1 m lasting perhaps for several millenia, followed by a rise in sea level to a height comparable to that associated with the first beach-ridge set. The second conclusion concerns the duration of these progradation phases. Well-dated Holocene barriers suggest that barriers of the size represented by the Pleistocene beach ridges seawards of the plan discontinuity at Evans Head require a minimum of 1,000 and 2,000 yr to form. Therefore, the New Guinea reef VIIb, infers that $\approx 120 \text{ kyr}$ high sea-level phase was 2,000 yr or longer, is supported, and curve A representing the Wilson surge-glaciation hypothesis is disqualified.

An additional test is to examine temperature changes over the same interval. According to the Wilson surge-glaciation hypothesis, temperature during the 120 kyr peak should be lower than during the $\approx 130 \text{ kyr}$ peak. A palaeo-temperature curve from rapid sedimentation-rate cores from the Gulf of Mexico¹⁶ is correlated in Fig. 1b with the sea-level envelope. Correlation of the temperature maximum with the $\approx 120 \text{ kyr}$ sea-level peak is consistent with modern deep sea core chronologies¹². Although a similar palaeotemperature record from higher latitudes would provide a more definite test—the Mexican Gulf perhaps being subject to temperature changes of a local rather than global type—this evidence again argues against the Wilson surge hypothesis. However, the facts discussed here do not preclude an alternative 'surge' hypothesis invoking disintegration of the West Antarctic ice sheet⁴ with consequent change of deep Atlantic circulation and mild equatorial warming leading, in some way, to initiation of northern glaciation.

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The sedimentary environment of the Deweras Group in Rhodesia

THE sediments of the Deweras Group comprise a thick succession of alluvial deposits laid down in a fault-bound trough and bear a close lithologic and tectonic similarity to the broadly contemporaneous sediments of the Waterberg, Soutpansberg and Matsap successions further to the south. It is significant, therefore, that the troughs in which all these sediments were deposited show a marked alignment and it is suggested here that they may be interpreted as a palaeo-rift system between 2,100 and 1,300 Myr old. The

Deweras Group (Figs 1 and 2) comprises a thick succession of clastic and argillaceous rocks, together with interbedded andesitic lavas. At the base of the sequence is a widespread but impersistent unit of continental tholeiitic basalts. These sediments crop out as a north-north-east (NNE) trending belt in central-north Rhodesia in a basin herein referred to as the Deweras trough.

The rock succession is predominantly arenaceous, comprising greywackes, subgreywackes, arkoses and feldspathic sandstones, together with poorly sorted conglomeratic units and thick interbeds of dolomitic argillite. The composition of the arenaceous units is dependent on the provenance area—arkoses and feldspathic sandstones being derived from granitic terrain, and greywackes and subgreywackes from the erosion of basement volcanics and metasediments. The clastics are typically poorly sorted and immature and quartz-arenites are absent from the succession. True conglomerates are lacking and most of these units are best described as sedimentary breccias. They are typically very poorly sorted, with adjacent clasts rarely in contact and embedded in a greywacke or dirty arkosic matrix. The clasts are strongly bimodal with rounded pebbles of quartz and granitic rocks, together with slivers of shale and phyllite. Some such units, 1–2 m thick, have been traced in a northeasterly direction for at least 1,500 m.

A feature of the clastic rocks is their rapid cyclicity. Graded bedding is the rule, and much of the succession comprises upward-fining alterations of sedimentary breccia, arkosic rocks and argillite, although upward-coarsening cycles are also encountered.

The thick argillaceous units are typically dolomitic, thinly bedded, and show strong intraformational deformation and enterolithic structures. Thin section analyses show anhydrite and gypsum to be intimately associated with such units, suggesting a very shallow-water penesaline depositional environment. Although bedded evaporites no longer exist, the presence of anhydrite and gypsum veins together with enterolithic deformation is evidence of their former

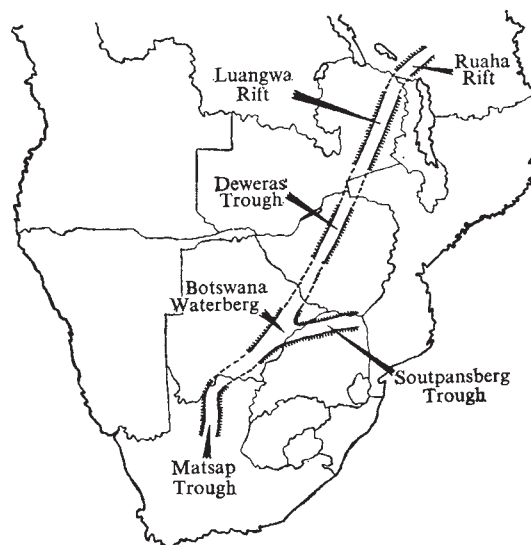


Fig. 2 Location of the Deweras and associated troughs in southern Africa.

existence. It seems, therefore, that the intraformational deformation of the argillaceous units is a result of load compaction due to the post-diagenetic movement (halokinesis) of thin anhydrite and gypsum interbeds. As dolomite forms part of the evaporite cycle, the thin dolomite interbeds probably represent chemical precipitates.

Throughout the success desiccation cracks, mudflake conglomerates, ripple marks, planar and trough cross-bedding, flute marks, flame-casts and scour channels are encountered—all indicative of a very shallow-water depositional environment subject to periodic desiccation.

As such, the sediments of the Deweras Group reflect many of the characteristics of alluvial deposits¹. The thin, laterally persistent conglomeratic units and their frequently argillaceous matrix show all the features of sheet-wash and debris-flows while the thick units of dolomitic argillite point to the presence of playa lakes in the centre of the basin. The poor sorting of the sediments, cut-and-fill structures, graded bedding, shallow-water structures and abundance of mudflake conglomerates also supports an alluvial origin.

These sedimentary features, together with the intra-cratonic position of the Deweras Group, as well as its elongate NNE-trending outcrop pattern all suggest that deposition occurred within an intracontinental rift system, dotted along its length by penesaline lakes. The sediments clearly do not reflect eugeosynclinal deposits^{2,3}.

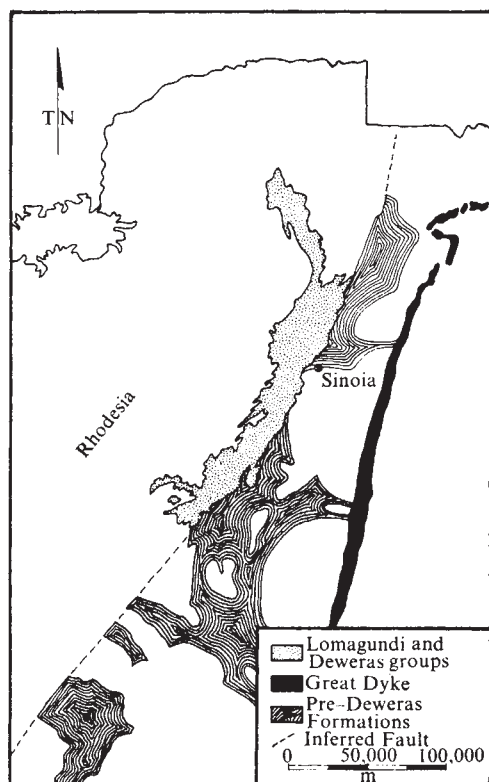
In view of the probability of deposition having occurred within an elongate, fault-bounded trough, it is interesting to note the abrupt truncation of older 'schist belts' (Fig. 1) along a line corresponding exactly to the southeasterly limit of the Deweras outcrop. This line must surely mark the position of the southeastern bounding fault of the Deweras trough. Such a tectonic setting would thus readily explain the rhythmic cyclicity of the succession as well as accounting for the interbedded volcanics.

Although the exact age of the Deweras succession is not known, it is certainly younger than the Great Dyke, and is unconformably overlain by sediments of the late Pre-cambrian Lomagundi Group. Thus, an age of between 2,000 and 1,300 Myr for the Deweras Group seems reasonable.

It is interesting to compare the Deweras succession with similar rock types in southern Africa. 'Red-bed' sediments of fluvio-continental origin cover wide areas of southern Africa where they are variously assigned to the Matsap, Waterberg and Soutpansberg Groups.

The sediments of these units have several features in common—they are predominantly arenaceous; shallow

Fig. 1 Distribution of Deweras and Lomagundi rocks in Rhodesia.



water structures persist throughout; they commonly show a strong linear trend; they are dated between 2,100 and 1,300 Myr. All these sediments show features of the classic 'molasse facies' of orogenic belts, yet, in the absence of such orogenies, cannot be interpreted as such. Within recent years, much attention has been paid to the depositional environment and tectonic setting of Waterberg and Soutpansberg rocks in the northern Transvaal⁷⁻⁸ and eastern Botswana⁹⁻¹¹.

Jansen⁵ interpreted Soutpansberg sedimentation as having occurred in an approximately east-west trending graben-structure which he has compared to an aulacogen. Crockett and Jones¹¹, in discussing the tectonic setting of Waterberg sedimentation in eastern Botswana, considered the sediments to have accumulated in fault-bound troughs which trend NNE, ENE and WNW to NW. It is noteworthy, therefore, that an extension of the ENE-trending graben structure of the Notwani Sector of eastern Botswana aligns itself perfectly with the Soutpansberg trough (Fig. 2). The NNE-trend is also important because it is the same direction as the Deweras trough in Rhodesia. Moreover, if extended to the SSW, the Deweras trough would meet the Soutpansberg trough in the area under consideration and may, in part, explain the complexity of block-faulting in eastern Botswana.

Although the Waterberg succession in the central Transvaal, around Nylstroom and Middelburg, is not so clearly part of a graben structure, Crockett^{10,11} suggested that deposition was influenced by the still actively subsiding Bushveld Basin which itself may have had the structure of a fault-bounded trough.

It is generally accepted^{4,11} that the Matsap and Waterberg Groups are broad chronostratigraphic equivalents. Jansen⁴ referred to the existence of a large sedimentary basin in early Waterberg times immediately east of Olifanshoek in the northeastern Cape which he termed the Matsap trough. It is surely not a coincidence, therefore, that this trough aligns itself directly with the eastern Botswana-Deweras sedimentary basins (Fig. 2).

Also, the NNE-trend of the Deweras Group in Rhodesia, when projected further to the NNE, aligns itself with the Luangwa Rift in eastern Zambia¹² and the Ruaha Rift in southern Tanzania.

Although the Luangwa Rift is filled with Karoo sediments this may not be significant as the graben structures both in Botswana¹¹ and the Soutpansberg⁵ have undergone tectonism up to and even after Karoo times.

Clearly, therefore, although this linear belt of fluvio-continental to lacustrine deposits extends throughout southern and central Africa and, for much of its length, marks a fossil rift valley system between 2,100 and 1,300 Myr, deposition need not and almost certainly was not coeval along the entire length. In fact, deposition seems to have occurred earliest in the south and latest in the north. Nonetheless, this belt did mark a zone of fundamental weakness within the Earth's crust, presumably an aborted attempt at continental drift.

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Observations of the deep-sea floor from 202 days of time-lapse photography

MANY photographic surveys have been made of the deep-sea floor to determine objects of interest to both geologists and biologists¹⁻⁴. With the exception of the use of the Isaacs⁵ baited 'Monster Camera', most photoreconnaissance surveys have suffered from being only instantaneous snapshots of the bottom. The added dimension provided by time sequence framing significantly increases the amount of information to be obtained from the use of bottom viewing cameras particularly in regard to the rates of various bottom processes. The Bottom Ocean Monitor (BOM) was developed by Gerard and Thorndike in 1974. It consists of a Thorndike time-lapse camera system and a long-term Thorndike photographic nephelometer mounted on a tripod with an Aandera current and temperature recorder⁶ above the tripod, all provided with an acoustic release. Using this equipment, we have taken more than 1,200 time-lapse photographs at 4,873 m depth in the North Equatorial Pacific Ocean. We report here that they show evidence of more rapid benthic biological processes and changes in sediment microtopography than had been previously assumed.

Pictures were obtained every 4 h; the nephelometer recorded at 2-h intervals; and the current speed, direction and water temperature were registered every 40 min. The camera which photographed an area of 1 m² was 1.6 m off the bottom with its axis inclined 30° to the vertical. From 6 September, 1976 to 25 March, 1977 this unit was positioned at latitude 11° 03.5'N, longitude 139° 58.9'W. This region is a known manganese nodule area and over 25 of these nodules are shown in the field of view of the 1,212 time-lapse frames. The most striking sequence of photographs began on day 34 and showed a wandering hemichordate, approximately 54 cm in length which moved at a speed of about 10 cm h⁻¹. The organism is either an enteropneust (acorn worm) or a lophenteropneust⁷. Hemichordates are commonly covered by a slime or mucous secretion^{7,8} which is considered protective and may also be used in food collection and/or digestion. Their body is divided into three easily recognisable parts—the proboscis, the collar, and the trunk. Between the eighth and twelfth hours of this series of photographs (Fig. 1) the hemi-

Fig. 1 Empty mucous tube less than 4 h after enteropneust's exit. Notice the trail at an oblique angle from the anterior (left) end.



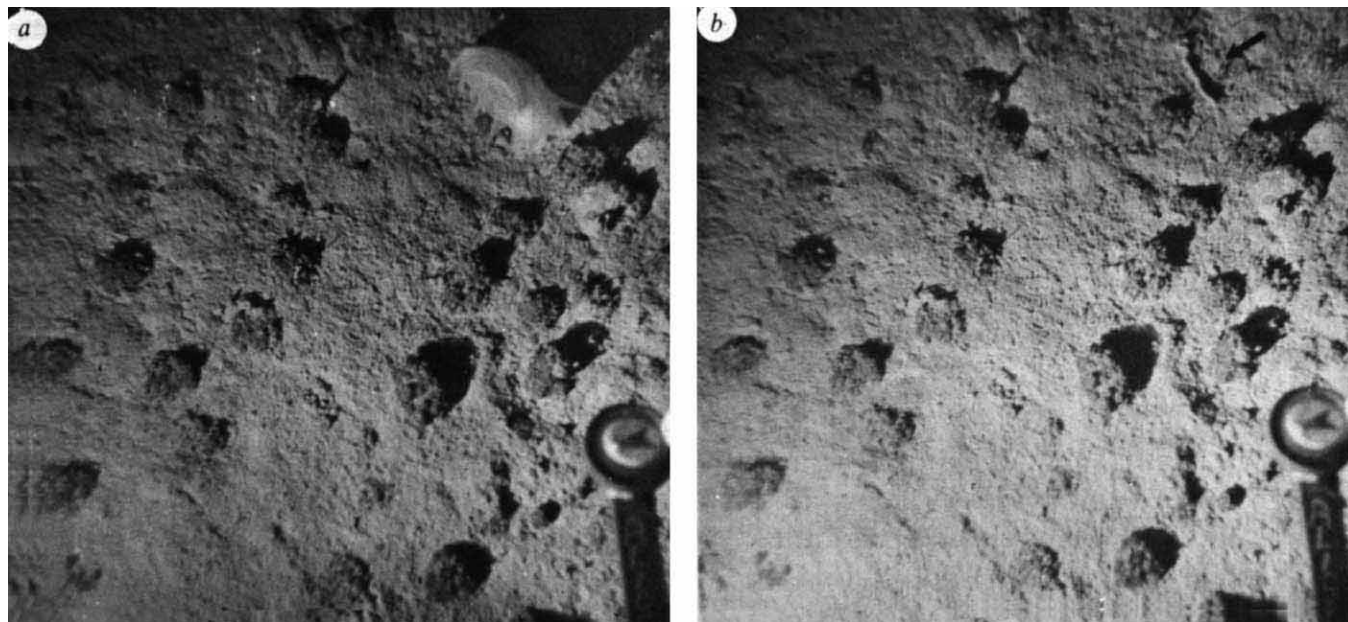


Fig. 2 *a*, Holothurian moving across field of view. *b*, Arrow points to faecal pile excreted by holothurian.

chordate shed or abandoned its mucous cover and then exited from the field of view. A likely explanation for this shedding, which has not been previously reported, is that the mucous cover is caught on the rough manganese nodules and as it can be reformed, the organism leaves it. The surprising observation that follows is the speed with which this mucous cylindrical sheath disappears. By day 6 there is only an outline left and by day 12 there is just a slight trace that remains on all subsequent photographs. Mucous is generally a polysaccharide or protein complex with high nutritional value and as we do not see any macrofauna feeding on it, or any sediment particles removed from nodule surfaces due to current activity, it is logical to assume that bacterial metabolism and meiofaunal activity are responsible for its rapid disintegration⁹. This observation is in contrast to the generally accepted view of extremely low rates of metabolism and consumptive decay stated by others as characteristic of the deep sea¹⁰⁻¹².

Faecal casts from holothurians are a common sight in bottom photographs and in the fossil records. The period for which they are thought to remain unchanged ranges from tens to thousands of years¹³. However, on day 65 an elaspod holothurian, probably of the genus *Peniagone*, entered the field (Fig. 2) and was gone 4 h later leaving a faecal cast. The cast is still discernable on day 202 but has noticeably degraded in size. The alteration is another indication that rates of biological activity in the deep sea can proceed much faster than previously imagined.

The process by which sediment on the ocean floor is mixed and moved by organisms living in it and on it is termed bioturbation. Bioturbation and the rate at which it takes place is of current interest to all researchers of the benthic boundary layer¹⁴⁻¹⁸. An example of bioturbation is furnished by the large anomuran crab (Fig. 3) that entered the field on day 87, leaving a trail of clear depressions caused by its walking appendages. Comparison of this fresh trail with the same tracks 115 d later (Fig. 4) shows the extent of change, presumably due largely to meiofaunal activity. The current velocity here rarely reaches its maximum of 9 cm s^{-1} and is not strong enough to cause sediment to move.

The importance of time-lapse photography to proper faunistic identification was emphasised on day 176, when four cylindrical forms appeared. In conventional deep-sea photography they would have been identified as holothurian faecal casts; however, in subsequent frames they moved and

changed shape. They are definitely organisms, although we cannot specifically identify them (Fig. 4).

As part of the Deep Ocean Mining Environmental Survey (DOMES) one of us (A.Z.P.) recently examined 7,863 photographs showing $81,355 \text{ m}^2$ of the deep-sea floor in the same general area of the ferromanganese nodule province of the North Equatorial Pacific that was studied here. That detailed examination of static scenes has shown 1,807 benthonots, with the following percentage composition: Holothuroidea, 35; Echinoidea, 16; Ophiuroidea, 13; Anthozoa, 19; Crustacea, 2; Porifera, Pennatul, Gorgonacea, Asteroidea, Ascidacea, Polyplacophora, Pynogonida, Echiuroidea, Gastropoda, Bivalvia, Bryozoa, Crinoidea, Polychaeta, and Enteropneusta made up the remaining 15. The density of these macrofaunal organisms was 3 per 100 m^2 . In the course of 202 d, the BOM photographed 1 Echinoidea, 1 Enteropneusta, 1 penaeid shrimp, 2 crabs, 5 other Crustacea, 8 Holothuroidea and 10 unidentifiable macrofaunal animals passing through the field of view. The similarity in density between the two investigations is quite impressive. The BOM photographed 35 macrofaunal animals in $1,212 \text{ m}^2$, for a macrofaunal density of 2.9 per 100 m^2 . Consideration of the fact that 25% of the DOMES

Fig. 3 Crab has just entered field. Note clear trail of small round depressions behind it.





Fig. 4 Same field of view 115 d after that in Fig. 3. Note that portions of the trail visible in Fig. 3 are completely erased and that the entire trail is less visible. Arrow points to unidentified organisms that resemble faecal casts.

organisms were sessile makes the comparison even closer.

The first long-term observation of the deep-sea floor serves to highlight how little is known of the benthic processes taking place there. We saw rapid apparent utilisation of a food source, although only slow rates had been previously hypothesised. Changes in faecal casts, formerly thought to take up to thousands of years, occurred in tens of days. Bioturbation appeared to change the microtopography of much of the field of view in a little over 6 months. The BOM has demonstrated its value on the deep-sea bottom; its continued use will be an aid in the examination of this presently difficult to study environment.

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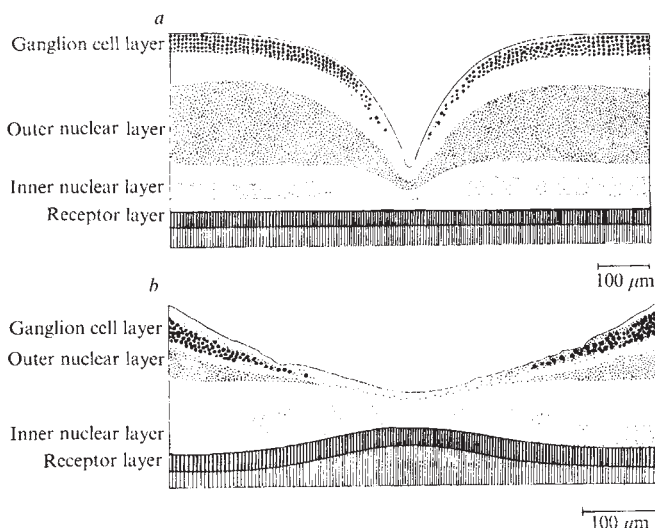
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The deep fovea as a focus indicator

MANY vertebrate eyes possess a depression (fovea) in the retinal tissue that overlies an area of receptor surface specialised for high acuity vision. The shape of this depression varies but two extreme forms have been characterised¹. Many birds and lizards² and some fish^{1,3} possess deep 'convexiclivate' foveas, while shallow bowl-like 'concaviclivate' foveas are characteristic of the eyes of some primates and the binocular field of some birds (Fig. 1). Various suggestions have been made to explain the existence and shape of these different foveal surfaces. The functions proposed have been mainly concerned with visual acuity and include reduction of chromatic aberration⁴, clearing of the retinal tissue to minimise image degradation (concaviclivate foveas)⁵ and image magnification (convexiclivate foveas)⁶⁻⁸. It has also been suggested that the deep convexiclivate fovea acts as a fixation device and/or movement detector at the expense of loss of visual acuity over a small area of image⁹. We show here how the deep convexiclivate fovea can act as a sensitive, directional focus indicator.

It has already been suggested that the convexiclivate fovea acts as a focus detecting spot analogous to those in modern reflex cameras to aid focusing the image¹⁰. There are several reasons for such a suggestion. (1) Convexiclivate foveas are placed symmetrically over retinal areas which have especially high receptor densities, which means that the area of image distorted by the foveal surface will lie in the centre of the high acuity field. This is the area of image from which it is obviously best to monitor the focus of the image and some evidence suggests that this is the case in humans (for review see ref. 11). (2) To avoid ambiguous signals from three-dimensional objects, it is better if information on the state of focus comes from a small area of the image. The dimensions and shape of the convexiclivate fovea are such that refraction at the surface will only affect a tiny area of the image of the order of 20 μm (10 receptors) diameter^{9,10}. Whatever the effect of foveal refraction on the image, the area involved is so small that its pictorial effect will be negligible but it can still act as a focus detector. (3) Any animal that has to change the focus of its eye has the problem of achieving an optimal focus setting. A case can be made, however, that the animals that have convexiclivate foveas are those that require a

Fig. 1 Diagrammatic cross sections of two types of fovea found in vertebrate eyes. *a*, The convexiclivate fovea is found in reptiles, birds and some fishes. *b*, The concaviclivate fovea is found in primates and the binocular field of some birds. The diagrams are modified from those of Polyak¹⁴.



special focus detector: the gross correlation between binocularity and concaviclivate foveas and monocularity and convexiclivate foveas can be explained in these terms. If an animal has binocular vision and a binocular system of distance estimation, this information can be used to cue the required lens accommodation to achieve a focused image for changes of distance of the point of fixation. Monocular animals, on the other hand, may have gross distance information less readily available and, if an animal relies on its focusing mechanism to estimate distance, as do chameleons¹², some special system for indicating the direction of focus error would be advantageous in achieving a rapid response. Furthermore, if an animal uses accommodation cues to estimate distance (and it seems likely that these cues are used by animals other than the chameleon) the accuracy of the estimate will depend on the accuracy with which the image can be focused. A focus detection spot, then, that provided high sensitivity focus information and/or a consistent criterion of 'focus' over a wide range of conditions would be of special use to such an animal (note the problems of night myopia that the human focusing system is apparently unable to correct¹¹).

To test the suggestion we used an Exakta VX 1000 camera. This has a removable focusing screen made of ground glass, which can be modified easily and reversibly. The screen was removed and a thick layer of nail varnish (Cutex colourless) was painted over a 5-mm diameter patch in the centre. The varnish was allowed to dry for 20 min and then a pin head was pressed into the centre of the spot causing a dimple shaped depression about 2 mm in diameter. After further drying, the screen was inserted into the camera.

The system has the following properties. When an image of a square pattern such as graph paper is formed in the normal plane, it is seen undistorted. If the lens is moved towards the screen, so that it focuses on a point beyond the object, marked barrel distortion is seen (Fig. 2a) and when the lens is moved forwards, so that it focuses on a plane closer than the object, pincushion distortion is seen (Fig. 2b). With practice, the camera objective (*f*/2 Zeiss Pancolor) can be set to within its normal depth of focus on any target that contains sharply defined pattern or outlines. The system gives particularly clear information about misfocus if the camera is not held steady and the 'fovea', which subtends about 2° at the observer's eye, gives a less ambiguous estimate of misfocus than the micropism screen with which the camera is supplied. The system functions in dim light.

The aerial image produced by the model fovea has been examined with a microscope which allows the image to be viewed with a shallow depth of focus. The image can then be seen at various levels; for example, it can be in focus and barrel distorted, out-of-focus and undistorted and still further out-of-focus and pincushion distorted. When the image is distorted, it has considerable astigmatism and the centre and periphery focus at different planes. When viewed thus, the depth of focus of the image is only a few micrometres but the distortion is still clearly visible in the out-of-focus image; it is not necessary then for the image to be focused for the mechanism to work.

The convexiclivate fovea has also been modelled diagrammatically (Fig. 3). It has been assumed that the lens has an aperture of *f*/4 (that of the fixating chameleon eye¹⁰) producing a cone of rays subtending $\pm 7.2^\circ$. The steepest angle of the side of the foveal pit is $\pm 10^\circ$ and the radius of the basal hemispherical region is 15 μm . The effective refractive index of the vitreous to retinal interface is taken as 1.0063, the value used by Walls⁶ based on the measurements of Valentin¹³. Ray plots for a central and an eccentric cone are shown for two planes of focus. The depth of focus of a *f*/4 lens at a resolution of 2 μm (the approximate diameter of the receptor cells) is about 15 μm . Accommodation of the

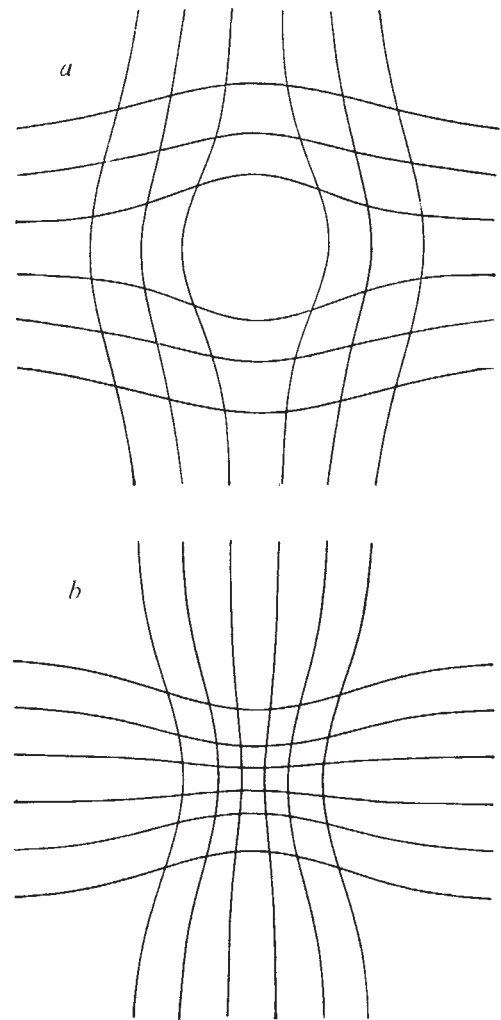


Fig. 2 Appearance of a regular squared graticule when viewed through a model fovea in a single lens reflex camera (for details see text) *a*, The camera lens is focused on a plane beyond the object and so the image is focused behind the model fovea. *b*, The camera lens is focused on a plane closer than the object and so the image is focused in front of the model fovea.

lens causing the extra-foveal image to move through this distance normal to the plane of the retina causes the axial foveal image to move through 80 μm and an image focused 7 min arc (6 μm) off the centre of the fovea to move through 35 μm . The fovea thus causes image misfocus to be amplified. Image distortion is also seen; a 15- μm change in the plane of focus of the extra foveal image causes about 2.3 μm lateral movement of the eccentric foveal cone shown in Fig. 3. This should be readily resolved, particularly if the image being focused spans several receptors.

The simple graphical model described here (Fig. 3) has dimensions similar to those of the chameleon fovea² and illustrates the feasibility of the mechanism. There are other aspects of the image distortion, not presented here, which may provide complementary information on focus error. The optimal design of foveal surface that is predicted by the model to maximise useful image distortion and minimise irregularities in the image plane will be discussed elsewhere. It is not necessary that the image should be undistorted when optimally focused as known distortions can be corrected centrally.

The magnitudes of these effects are sensitive to the steepness of the sides of the foveal pit and the relative refractive indices of vitreous humor and retina. The distortion and flatness of field in the fovea are sensitive to the shape of

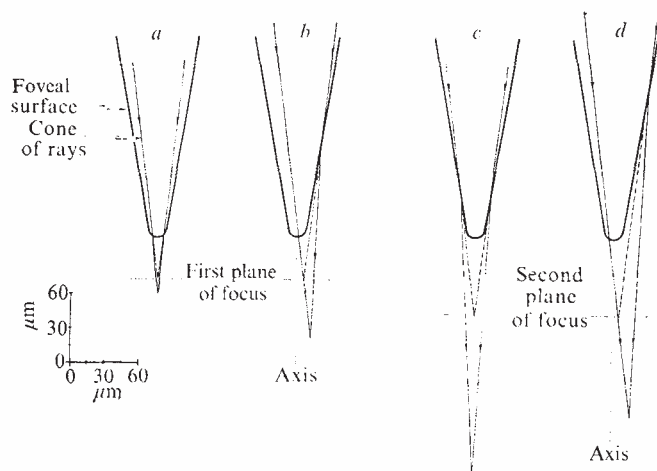


Fig. 3 Ray plots of cones of light passing through a model fovea; for details of the model see text. In the absence of the fovea, the cones of rays would focus on the planes indicated by horizontal dashes; in (a) and (b) this plane is 30 μm closer to the lens than in (c) and (d). In (a) and (c), cones passing through the axis of the fovea are focused on planes 160 μm apart, for a 30- μm change in the plane of focus of the lens. In (b) and (d) rays passing along an axis 6 μm eccentric to the axis of the fovea focus on planes 70 μm apart for a 30- μm change in the plane of focus of the lens and show a 4.5- μm lateral shift of the centre of the cone for a 30- μm change in the plane of focus of the lens.

the base of the foveal pit and the distance of the effective receptor surface from the base of the pit.

Hitherto, investigation of the convexitivate fovea has concentrated on its possible function in improving visual acuity and the effect of foveal refraction on the image may well be to improve an animal's performance in tasks designed to test visual acuity even if the image is not focused. However, as there is circumstantial evidence suggesting that the convexitivate fovea is concerned with focus detection and as the models presented here show that refraction at the foveal surface could provide sensitive unambiguous information on focus error, we suggest that the function of the deep fovea as a focus indicator may be of greater biological importance.

We thank Dr M. F. Land for helpful discussion of our models and Jonathan Erichsen for discussion of results of his computer simulations of ray paths through model foveas; these show similar properties to those described here.

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Ocular motility and recovery of orientational properties of visual cortical neurones in dark-reared kittens

THE specific orientational properties of visual cortical neurones originally described by Hubel and Wiesel¹ in adult cats have also been observed in normally reared kittens²⁻⁴. Such properties are lacking, however, in kittens reared in complete darkness for six weeks³⁻⁵. Three types of cells, specific, immature and nonspecific, have been defined by Imbert and Buisseret⁴ in kittens. In dark-reared (DR) kittens, a few hours of visual experience are sufficient to elicit a rapid increase in the proportion of orientation-specific cells^{3,5,6}. We report here a series of experiments undertaken to determine the factors involved in the 'reconstructive effect'⁷ of visual experience for cortical specificity, and show that oculomotor exploration, as well as visual input, is an essential factor.

In the first series, six DR kittens (six weeks old) were exposed to a normal visual environment for six hours. For four of them (group a) the responses of cells in area 17 were studied electrophysiologically immediately after the period of active visual experience. Three types of visual cell were found; 94% had an orientated receptive field but 82% were immature, responding over a very broad range of orientation (Fig. 1, column II). In four kittens (group b), which were put back in the darkroom for 12 h after the period of exposure to light, the distribution of cells was altered (Fig. 1, column III) and became closer to that of normally reared (NR) kittens (Fig. 1, column VI) but with a higher proportion of immature cells. However, a χ^2 comparison test applied to the cells with orientational properties, that is, immature and specific, corrected for small samples (Yates), indicates no significant differences between group b and normal kittens.

The percentage of specific cells was much higher in group b than in group a, 45% compared with 12%. It seems then that if a period of 6 h of visual experience is

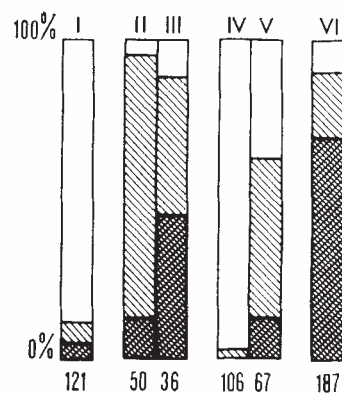


Fig. 1 Distribution in % of the three types of visual cortical units (area 17) recorded after different visual exposures in six-week-old dark-reared kittens. Cells were recorded with metallic microelectrodes. The kittens were anaesthetised, curarised and artificially ventilated. Contact lenses protected their corneae from drying. Visual stimuli, slits or spots, were displayed on a tangent screen. Columns: I, dark-reared, VI, normally reared kittens. During 6 h of exposure, conditions were: II and III, freely moving; IV, paralysis; V, restraint. In III, IV and V, 12 h in the dark followed the 6 h of exposure. Numbers of visual cells recorded are given under each column. Specific cells (▨) are activated by orientated stimuli within a sharp angle ($<60^\circ$). Immature cells (▤) are activated by orientated stimuli within a larger angle ($<150^\circ$). Nonspecific cells (□) are activated by non-orientated stimuli moving in any direction.

sufficient to allow cortical cells to recover orientational properties, the return to the darkroom for 12 h without any further visual experience facilitates the formation of orientational selectivity, confirming former observations⁸. It is proposed to call the latter period one of 'consolidation'.

Four DR kittens were exposed to light for 6 h but were paralysed by curarising drugs. They were artificially ventilated and their electrocardiograph and electroencephalograph (EEG) were routinely monitored; their EEG records indicated a good level of arousal. It was periodically checked that the diameter of the pupils and the projections of the visual axes were in optimal condition for good retinal images. Two kittens were paralysed with gallamine (Flaxedil) and two with *d*-tubocurarine (which, because of its high molecular weight, is presumed not to cross the blood-brain barrier). One animal of each pair was exposed to an illuminated view of people moving around the room (1–3 m), while the other was passively moved around in the laboratory. At the end of the six-hour period, all the kittens, after they had recovered their spontaneous respiration, were put back in the darkroom for 12 h of 'consolidation'. As no difference was noted among the four kittens, the proportions of the three types of visual cortical units were represented by a single column (Fig. 1, column IV). Very few cells with orientational properties were observed, in contrast to the data of Pettigrew and Garey⁸, whose experimental conditions at least for one of their kittens were not very different from ours. A χ^2 test showed no significant difference between the DR and the paralysed kittens. These observations show that passive exposure to a normal visual environment, by itself, does not induce orientation specificity, and suggest that visuomotor interactions are necessary for the restoration of this specificity.

A third type of experiment was designed to investigate the role of ocular motility. The general motility of four DR kittens was reduced by enclosing their heads and bodies in plaster casts in such a way that only exploration by ocular movements was possible during the period of exposure to light; this period was followed by the usual 'consolidation' period. Two kittens were passively carried around the laboratory while two others were held stationary with a view of moving people. As no difference was noted between the two sets of animals, the proportion of the three types of cortical units found in these kittens was calculated for the four animals (see Fig. 1, column V). In striking contrast to paralysed animals, the majority of the visual cells had acquired orientational specificity. Statistical analysis showed that the distribution of cells in kittens with ocular movements alone differs from the distribution observed in freely moving animals at only the 0.1 level of significance (Fig. 1, column III). Thus, provided ocular motility is preserved, the suppression of body movements reduces, but does not prevent, the restoration of orientation specificity, whereas the abolition of all movements does prevent it.

Altogether, these observations demonstrate that ocular motility plays an essential part in the 'reconstructive effect'⁷ of visual experience for orientational properties. Projection to the striate cortex of impulses generated by stretching extraocular muscles has recently been demonstrated⁹. It is thus conceivable that information provided by proprioceptive receptors belonging to these muscles participates in the specification of visual cells.

In conclusion, the orientational properties of visual cortical cells, which have disappeared (compare refs 4, 10) in six-week-old dark reared kittens, can reappear after a 6-h period of active visual exploration. However, orientation selectivity similar to that of NR kittens is restored only when there is a complementary period of 'consolidation'. Reduced but significant recovery is still observed when only the eyes are free to move. It is suggested that proprioceptive afferents from extraocular muscles which project to the

visual cortex⁹ play a part in the acquisition of the orientational properties of visual cortical neurones.

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Pheromonal chirality and integrity of aggregation response in southern species of the bark beetle *Ips* sp.

PHEROMONAL communication by which southern pine engraver beetles signal aggregation and colonisation involves three terpene alcohols, ipsdienol (2-methyl-6-methylene-2,7-octadien-4-ol), ipsenol (2-methyl-6-methylene-7-octen-4-ol) and *cis*-verbenol first isolated from *Ips paraconfusus* Lanier in California¹. The absolute configurations of these compounds are (1*S*, 4*S*, 5*S*)-*cis*-verbenol², (S)-(–)-ipscenol³, and (S)-(+)-ipscenol⁴. *I. grandicollis* (Eichhoff) responds to (S)-(–)-ipscenol⁵ and seems to produce it as its only pheromonal compound. *I. calligraphus* (Germar) uses (S)-*cis*-verbenol and ipscenol as an aggregation pheromone, and *I. avulsus* (Eichhoff) aggregates in response to ipscenol and (S)-(+)-ipscenol, apparently the combination of a pheromone and a kairomone⁶. Although frass produced by *I. paraconfusus* attracted *I. grandicollis* in field tests, it failed to aggregate either *I. calligraphus* or *I. avulsus*. Similarly, *I. calligraphus* producing (S)-*cis*-verbenol and ipscenol did not aggregate *I. paraconfusus* when frass of *I. grandicollis* was added as a source of (S)-(–)-ipscenol. Laboratory bioassay has confirmed the lack of cross attraction between *I. paraconfusus* and *I. calligraphus*⁷. Furthermore, when synthetic pheromones were used in field bioassay, *I. avulsus*, *I. grandicollis* and *I. calligraphus* readily responded to racemic formulations of their own pheromones, but there has been only one report of field attraction of *I. paraconfusus* with racemic pheromonal components⁸. We now offer an explanation for these phenomena which involves the field responses of southern *Ips* sp. to the enantiomers of ipscenol⁹.

Tests were performed near the Lufkin laboratory of the Pest Control Section, Texas Forest Service, where a heavily forested area of predominantly *Pinus taeda* L. maintains an endemic

Table 1 Field responses of *Ips calligraphus* to sleeve olfactometers baited with (S)-*cis*-verbenol and the enantiomers of ipscenol

Test material: (S)- <i>cis</i> -verbenol* and	Replications	Average no. (and range) of beetles responding	Sex ratio ♂:♀
(–)-Ipsdienol*	4	9.5 (4–13)	1:1.7
(+)-Ipsdienol*		1.3 (0–2)	1:1.5
(–)-Ipsdienol*	4	8.8 (5–16)	1:2.3
Ipsdienol (rac)†		4.5 (1–9)	1:3.5

Data obtained in Lufkin, Texas, 19–20 July; test duration, 30 min.

*Estimated average release 2 mg h⁻¹.

†Estimated average release 4 mg h⁻¹.

Table 2 Field response of *Ips avulsus* to sleeve olfactometers baited with racemic ipsenol and the enantiomers of ipsdienol

Test material: Ipsenol† and	Replications	Average no. (and range) of beetles responding	Sex ratio ♂ : ♀
(-)-Ipsdienol*	4	73.3 (43-98)	5.1 : 1
(+)-Ipsdienol*		13.8 (6-26)	1.3 : 1
(-)-Ipsdienol*	4	11.0 (3-23)	19.5 : 1
Ipsdienol (rac)†		4.3 (1-7)	3.3 : 1

Data from Lufkin, Texas, 25-27 July; test duration, 30 min.

*Estimated average release 2 mg h⁻¹.

†Estimated average release 4 mg h⁻¹; vial 4 mm inner diameter × 50 mm.

population of engraver beetles. Two fan-driven sleeve olfactometers¹⁰ set 6m apart served as traps. If not noted otherwise, test materials were placed in vials 50 mm long and 9 mm inner diameter and closed with plastic stoppers perforated with two holes about 1mm diameter to allow for slow evaporation. Separate vials were used to allow for release of approximately 2× of the racemic materials when these were tested against the single enantiomers. In this way, the active enantiomer was assumed to be evaporated at a level comparable to the optically pure compound. Responding beetles were collected from the canvas sleeves of the olfactometers as they landed. After each test, the materials were switched between the two olfactometers to avoid possible positional effects. Compounds tested were racemic ipsenol (94%), racemic ipsdienol (96%), both stabilised with hydroquinone; (*S*)-*cis*-verbenol (94% optical purity, Borregaard Industries); (*R*)-(-)-ipsdienol (90% optical purity, Firmenich) and (*S*)-(+)-ipsdienol (80% optical purity, Firmenich).

Both *I. calligraphus* and *I. avulsus* responded to (*R*)-(-)-ipsdienol (Tables 1 and 2) and therefore might be expected to produce that compound. This is in accord with the lack of attraction of *I. calligraphus* to *I. paraconfusus* in California which has (*S*)-(+)-ipsdienol in its pheromonal communication system. *I. calligraphus*, *I. grandicollis* and *I. avulsus* are common pests of pine trees in the southern and south-eastern United States, frequently inhabiting the same tree or those colonised by the southern pine beetle, *Dendroctonus frontalis* Zimm. *I. calligraphus* also occurs in *Pinus ponderosa* forests at low elevations of the west slope of the central Sierra Nevada in California, where it occurs sympatrically with *Ips paraconfusus* Lanier and *Dendroctonus brevicomis* LeC¹¹. The respective pheromonal systems known so far are summarised in Table 3. Only *I. grandicollis* is cross-attractive to allopatric *I. paraconfusus*.

We suggest that the lack of response to the tripartite *I. paraconfusus* pheromone by *I. calligraphus* and *I. avulsus* results from species-specific differences in response to the optical antipodes of ipsdienol, that is, the absence of the attractant enantiomer as well as the presence of the antipode which may

cause response inhibition⁷. It seems that (*S*)-(+)-ipsdienol may somewhat suppress the response of *I. calligraphus* and *I. avulsus* to (*R*)-(-)-ipsdienol when both enantiomers are offered together (Tables 1 and 2). Similar effects have been observed in other cases where the response to the attractant enantiomer of a pheromonal compound is inhibited by the 'inactive' antipode^{12,13}. In a significant case, field tests with racemic and optically pure isomers of ipsenol and ipsdienol indicated that the response of *I. paraconfusus* to synthetic chemicals was readily blocked by the addition of (*R*)-(-)-ipsdienol and (*R*)-(+)-ipsenol, both antipodes of the natural pheromonal components (J.P.V., unpublished). The failure of racemic compounds to attract *I. paraconfusus* populations in the field had been attributed to a hypothetical delicate dose-response relationship rather than response inhibition by the antipodes of pheromonal attractants.

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Immunosuppression by *Babesia bovis* against its tick vector, *Boophilus microplus*

INFECTION with protozoan parasites may interfere with the immune response to a variety of agents including bacteria¹, other protozoans²⁻⁵ and helminths⁶⁻⁸. In two recent studies of immunosuppression by a rodent piroplasm^{8,9}, the likely, veterinary importance of the effect was suggested. We have now verified this possibility by showing that *Babesia bovis*, a pathogenic piroplasm of cattle causes immunosuppression against its natural tick vector, *Boophilus microplus*. The observation seems to be the first of immunosuppression caused by a protozoan against a parasitic arthropod.

B. microplus normally spends all its parasitic life (approximately 21 d) on one host. Partial acquired resistance can be produced by exposing the host to ticks and is manifested by a reduction in the number of ticks maturing¹⁰. In two experiments, we applied 0.25 g (5,000) tick larvae bi-weekly, to animals for up to 25 weeks. Mature ticks dropping every day from groups of animals were collected and counted, and the numbers used to measure the level of host resistance. In the second experiment, ticks >4.0 mm in length still attached to the host were also counted¹¹, and the values used as a measure of resistance of individual animals.

In experiment 1, two groups of four calves (cross-bred *Bos taurus*) were placed in separate isolation pens when the calves were 1 month old, and infested with larvae as described. At day 21, when the first adult ticks were falling, one group was inoculated intravenously with 10⁶ *B. bovis*, attenuated for vaccine¹². Because they were attenuated, the *B. bovis* produced only a low level primary parasitaemia detectable for a maximum

Table 3 Pheromone systems communicating species-specific aggregation of engraver beetles sympatric to California (a) and Texas (b) pine forests

Engraver species (pheromonal system)	Known components of aggregation pheromone and assumed configuration	Field response
<i>I. paraconfusus</i> ^a (Tripartite)	(<i>S</i>)- <i>cis</i> -verbenol (<i>S</i>)-(+)-ipsdienol (<i>S</i>)-(-)-ipsenol	<i>I. paraconfusus</i> <i>I. grandicollis</i>
<i>I. calligraphus</i> ^{a,b} (Dual component)	(<i>S</i>)- <i>cis</i> -verbenol (<i>R</i>)-(-)-ipsdienol	<i>I. calligraphus</i>
<i>I. grandicollis</i> ^b (Single component)	(<i>S</i>)-(-)-ipsenol	<i>I. grandicollis</i>
<i>I. avulsus</i> ^b (Pheromone/Kairomone)	(<i>R</i>)-(-)-ipsdienol (plus (<i>S</i>)-(-)-ipsenol)*	<i>I. avulsus</i>

*Natural source, *I. grandicollis*.

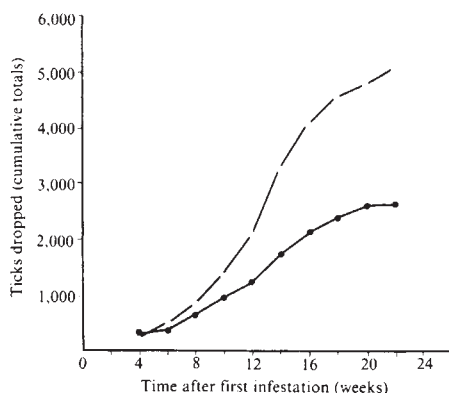


Fig. 1 Tick infestations with *B. microplus* expressed as average cumulative totals of all ticks dropped from four calves infected at day 21 with *B. bovis* (□) and four calves not infected (●). Fewer ticks mean greater resistance.

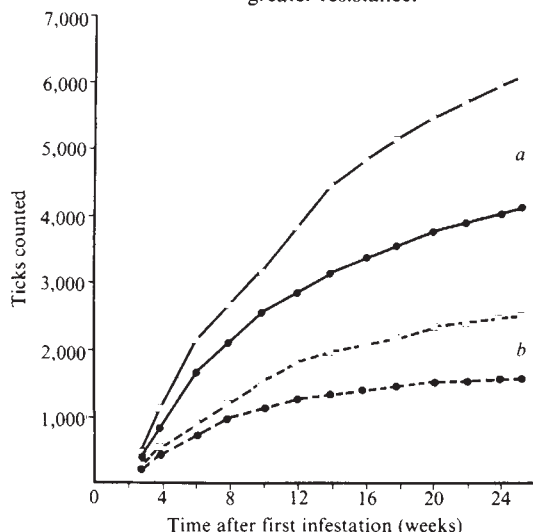
of 3 d and no fever, in all the calves used. Figure 1 shows that over a period of 22 weeks, total tick production was much greater in infected than in uninfected calves.

Experiment 2 differed in several ways from experiment 1: six calves were inoculated with *B. bovis* 4 d before the first batch of tick larvae were applied to these and to a further six uninoculated animals; a more virulent strain of *B. bovis* was used, which necessitated babesicidal treatment to control primary infections; bi-weekly body counts of ticks on all 12 calves were made, providing individual values suitable for statistical analysis.

Intravenous inoculations of 10^8 *B. bovis* produced moderate parasitaemias associated with fever, beginning 1 d before the first application of ticks. One day after the first ticks were applied all calves (including the six uninoculated animals) received 0.5 ml 5% quinuronium sulphate, which suppressed the primary infections.

Figure 2 shows that over a period of 25 weeks, resistance to ticks, based on two different measurements of the numbers maturing, was greater for the uninfected than for the infected animals, confirming the immunosuppressive effect of *B. bovis*.

Fig. 2 Tick infestations with *B. microplus* expressed as average cumulative totals of: a, all ticks dropped (—) and b, bi-weekly body counts (ticks >4 mm) (---) of six calves infected (□) with *B. bovis* at day 4 and six left uninfected (●). Differences became significant ($P < 0.05$) for b at week 13, significance subsequently increasing. Fewer ticks counted mean greater resistance.



Although this effect was less pronounced than in experiment 1, analysis of variance of data derived from body counts showed that the significance of the difference increased with time, becoming $P < 0.05$ at week 13.

We have thus shown that a primary infection with *Babesia* at about the time of first exposure of the host to *B. microplus* will alter, perhaps permanently, the level of resistance acquired by the host to this tick. Data plotted in Figs 1 and 2 show that more ticks were maturing on infected than on uninfected cattle at the end of the observation periods.

This finding is of epidemiological interest, and is also relevant economically (sometimes *B. microplus* causes greater loss than babesiosis¹³). Cattle becoming infected with *Babesia* either naturally or by vaccination¹² at the time of their first tick infestation may subsequently suffer more from tick worry than those that escape babesial infection. This undesirable synchronism should be avoided whenever possible. The other epidemiological aspect is that by reducing host resistance to ticks, *Babesia* may improve its own chance of survival, its prevalence being related to vector density¹⁴.

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Isozymic heterogeneity of *Trypanosoma cruzi* in the first autochthonous patients with Chagas' disease in Amazonian Brazil

CHAGAS' disease, in which the aetiological agent is *Trypanosoma cruzi*, is a principal cause of morbidity and mortality on the South American continent; at least 10 million people are said to be infected¹. There is no vaccine or acceptable chemotherapy², and insecticide control of domiciliated triatomine vectors (*Hemiptera, Reduviidae*) remains problematical. Human survivors of the acute phase of infection commonly develop electrocardiographic abnormalities, chronic cardiomyopathy and singular chagasic syndromes consisting of cardiomegaly, megacolon and megaesophagus. *T. cruzi* from diverse mammals and vectors are morphologically indistinguishable³, and are prudently treated as a single entity pathogenic to man. However, heterogeneity has been suspected⁴ from behaviour in experimental hosts, discordant therapeutic trials, and, most notably, from both the enigmatic, disparate geographical distribution of chagasic syndromes among infected populations⁵ and the different descriptions of the pathogenesis of the heart disease^{6,7}. By means of enzyme electrophoresis, we show here, for the first time, the heterogeneity of *T. cruzi* infecting man.

Since its development⁸, enzyme electrophoresis has pro-

vided a means of indicating molecular differences among specific genetic products of organism populations and has had widespread application, particularly in the field of human genetics^{9,10}. By this method, we have identified distinct sylvatic and domestic strain-groups of *T. cruzi*, apparently circulating independently and transmitted by different vector species, in the rural town of São Felipe, Bahia state, eastern Brazil¹¹. One strain-group, which we now call type I, occurred in opossums (*Didelphis azarae*) and a sylvatic triatomine species (*Triatoma tibiamaculata*); the second type (type II) was found in acute and chronic cases of Chagas' disease, cats, house mice, rats and guinea pigs, from houses infested by the domiciliated triatomine *Panstrongylus megistus*. There was no evidence that the

separation of the two forms depended on an inability of the sylvatic form to infect man, as has been suggested by analogy with the *T. brucei* subspecies causing human trypanosomiasis in Africa¹. Using electrophoretic mobilities of six enzymes (E.C.1.1.1.40; E.C.1.1.1.49; E.C.2.6.1.1; E.C.2.6.1.2; E.C.2.7.5.1; E.C.5.3.1.9, refs 12, 13), and methods already described¹¹, we have since compared type I (sylvatic) and type II (domestic) *T. cruzi* from São Felipe with *T. cruzi* responsible for six of the first seven acute cases of Chagas' disease recorded in the Amazonian city of Belém, Pará state, 1,600 miles north-west of São Felipe.

All six of the Belém cases were of local origin, and thought to be derived from *T. cruzi*-infected sylvatic vectors, which have now been found in mammal nests within 25 m of houses in the city (M.A.M. *et al.*, unpublished work). Our results (Figs 1 and 2) demonstrate that two of the six cases were due to *T. cruzi* indistinguishable from the sylvatic, type I *T. cruzi* of São Felipe, previously unknown in man¹¹. The other four cases were simultaneous within a single household, and thought to have been acquired by oral transmission¹⁴; they were all due to a third form of *T. cruzi*, which we now call type III, with enzyme mobilities as explained in Figs 1 and 2. Characterisation of over 60 stocks¹¹ of *T. cruzi* from sylvatic mammal and vector species demonstrate that type I *T. cruzi* predominates, and that type III *T. cruzi* is occasionally found, in the enzootic cycles of the surrounding Amazonian forest (M.A.M. *et al.*, unpublished work). We conclude that at least three discrete forms of *T. cruzi* do indeed infect man in Brazil; all three can cause acute Chagas' disease, and at least one (type II) causes chronic chagasic syndromes in Bahia state.

The types of *T. cruzi* in man, in both Belém and São Felipe, accord with local cycles of transmission. With

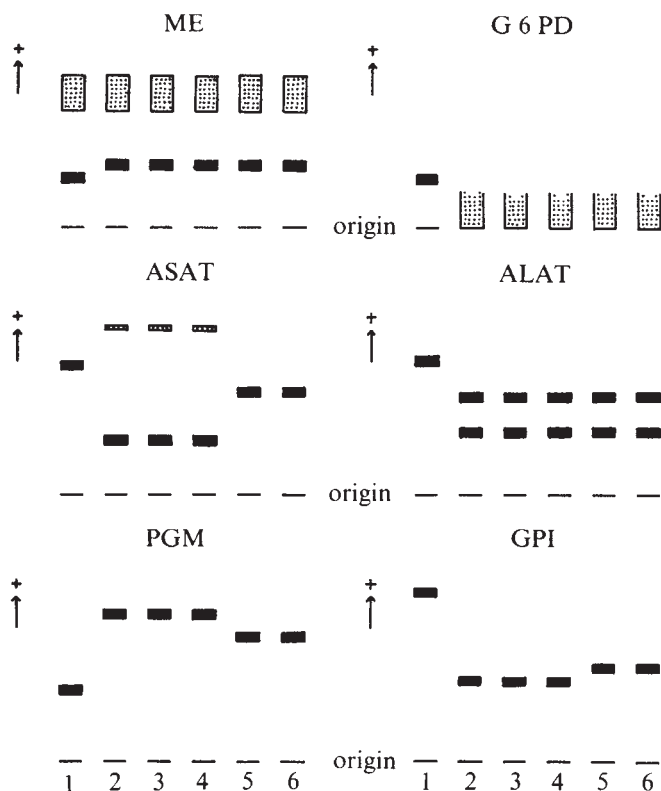
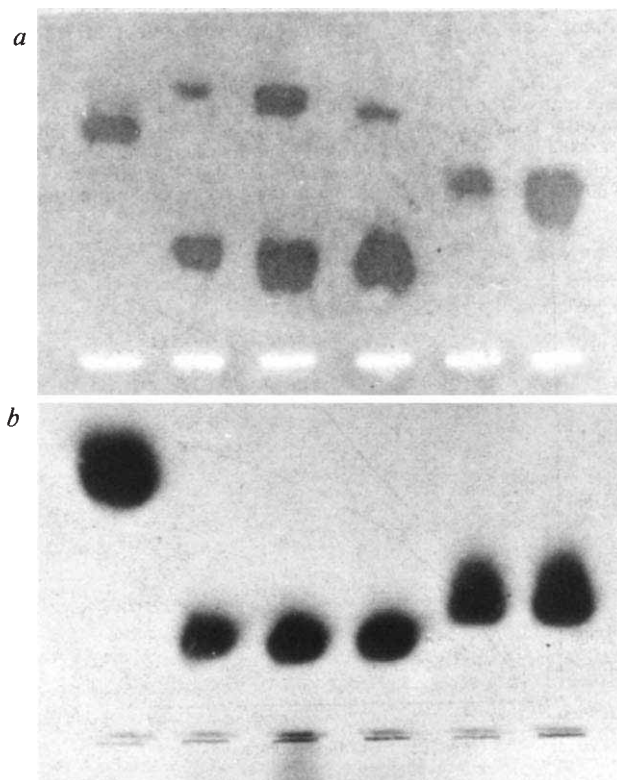


Fig. 1 Comparative electrophoretic patterns of culture forms of *Trypanosoma cruzi* types I, II and III. The six enzymes shown are: E.C.1.1.1.40 (refs 12, 13), malate dehydrogenase (oxaloacetate-decarboxylating) (NADP⁺), ME ('malic' enzyme); E.C.1.1.1.49, glucose-6-phosphate dehydrogenase, G6PD; E.C.2.6.1.1, aspartate aminotransferase, ASAT; E.C.2.6.1.2, alanine aminotransferase, ALAT; E.C.2.7.5.1, phosphoglucosmutase, PGM; E.C.5.3.1.9, glucosephosphate isomerase, GPI. Prominent bands are shown in black; faint or diffuse bands are stippled; weak, fast-moving bands on ASAT and satellite bands on PGM are omitted; type I and III G6PD is diffuse and streaks from the origin. Methods are as described by M.A.M. *et al.*¹¹. Reference standards (in this case, 1 and 2 below) are included on every plate, as fluctuations in electrophoretic conditions alter distances from the origin. In each case enzyme extracts are, from left to right, of *T. cruzi* stocks derived from the following hosts: 1, man, acute case, São Felipe, Bahia, Brazil (SF/BAHIA/71/Esmeraldo/119) (ref. 11), type II; 2, opossum, São Felipe, Bahia (SF/BAHIA/75/*Didelphis azarae* WA250/133) (ref. 11), type I; 3, opossum, Utinga reserve, Belém, Pará, Brazil (PARA/77/*Didelphis marsupialis*; Linha 5/47), type I; 4, man, acute case, Belém, Pará (PARA/77/M4628/43), type I. Type I pattern was also shown by *T. cruzi* from 1 other of the 6 Belém cases examined (PARA/72/M1815/94); 5, *Panstrongylus lignarius*, Utinga reserve, Belém, Pará (PARA/77/Bug 21/101), type III; 6, man, acute case, Belém, Pará (PARA/68/CAN III/198), type III. Type III patterns were also shown by *T. cruzi* from three other simultaneous acute cases in the same family¹⁴. Type I is quite distinct from type II on all six enzymes; types I and III differ only on ASAT, PGM and GPI. Thus *T. cruzi* type I, previously known only from the sylvatic cycle in São Felipe¹¹ and type III, not found in São Felipe, are responsible for the first autochthonous cases of acute Chagas' disease recorded in Amazonian Brazil. Scale: the cotton thread carrying each extract at the origin measures about 0.5 cm.

Fig. 2 Photographs of zymograms of ASAT (a) and GPI (b) showing enzymic types I (extracts 2-4) II (extract 1) and III (extracts 5 and 6) of *Trypanosoma cruzi*. Extracts are, from left to right, of the same stocks as given in Fig. 1. Methods: ASAT, 450 V, 120 min; GPI, 300 V, 180 min, otherwise as described by Miles *et al.*¹¹. Scale—cotton thread at the origin measures about 0.5 cm.



certain provisos⁴, it is likely that the differences established here have a genetic basis and are persistent¹⁵. We have used standardised procedures¹¹ and, as controls, type I and type II *T. cruzi* from São Felipe have remained stable for over two years during numerous passages, in four different culture media; enzyme patterns were also independent of original hosts. In further, preliminary experiments we have not as yet been able to show interaction of type I and type II *T. cruzi* in mixed culture, with the emergence of new enzyme patterns; although genetic exchange is unknown in trypanosomes, such interactions in mixed infections in the vertebrate or invertebrate hosts cannot be totally excluded. From our results we are optimistic that enzyme markers will correlate with biological characters and the distribution of the human disease, and lead to a more rational selection of *T. cruzi* used for chemotherapeutic and immunological comparisons.

The presence of enzootic *T. cruzi* (types I and III), capable of causing acute and perhaps chronic Chagas' disease, threatens the human population of the Amazon basin. Fortunately, none of the seven sylvatic vector species captured to date around Belém (ref. 16 and M.A.M. *et al.*, unpublished work) are known to colonise houses⁴. Nevertheless, many local mud and wattle or palm-roofed houses seem ideally suited to triatomine infestation, and notorious domiciliated vectors, such as *Rhodnius prolixus* and *Triatoma infestans*, thrive in secure laboratory colonies in Belém. Thus, the greatest risk of endemic Chagas' disease to the Amazon basin seems to be from the arrival, from other endemic areas, of known house-adapted vector species carrying *T. cruzi*, and not from the colonisation of houses by local bugs.

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Cell saturation density is not determined by a diffusion-limited process

THE mouse cell line 3T3 ceases cell division at a confluent saturation density despite regular medium replacement. Also, when a confluent quiescent monolayer of 3T3 is wounded, cells migrate into the wound free of cell contact and initiate DNA synthesis, while cells in the monolayer remain arrested¹. As the cells in both areas are in the same bulk medium, this experiment proves that depletion of growth factors in the bulk medium is itself not sufficient to account for density-dependent inhibition of growth. Two hypotheses have been proposed to explain the wounding experiment. The first states that direct cell-cell contact, perhaps involving the interaction of a receptor with its ligand on an adjacent cell, provides a negative signal for growth¹. The second hypothesis states that growth ceases at a confluent cell density because of the establishment of a diffusion boundary layer which maintains the supply of one or more growth factors below a critical level². A diffusion boundary layer results because convective movement of liquids near a solid surface approaches zero. In these conditions, the rate of delivery of molecules to and from the surface is limited by the rate of diffusion through the boundary layer. Increased fluid velocity on confluent cells causes initiation of DNA synthesis^{2,3}, a finding apparently supporting the diffusion barrier hypothesis. Theoretically, increasing the fluid velocity over the cell layer should decrease the thickness of the hypothetical boundary layer and therefore increase the rate of delivery of a diffusion-limited growth-controlling substance⁴. However, in these experiments it was not possible to rule out an effect of fluid velocity on cell-cell contact. Yet if growth is a diffusion-limited process, then increasing viscosity of the cell culture medium should decrease the saturation density. We report here that increased viscosity has no effect on saturation density or on the rate of cell growth, providing strong evidence against the diffusion barrier hypothesis.

Swiss 3T3 cells were plated at a subconfluent density (Table 1) in 10% serum. 24 h later the medium was changed to 10% serum, 5% serum and 2% serum. Other dishes

Table 1 Effect of viscosity on saturation density

Treatment	Cells per dish ($\times 10^4$)		Relative viscosity
	Day 3	Day 5	
10% Serum	77.8	79.3	1
5% Serum	45.4	41.3	—
2% Serum	24.9	24.0	—
10% Serum plus:			
10% dextran	75.8		20.6
10% Ficoll	77.1		4.8
2% methylcellulose	81.4		25.0

Swiss 3T3 cells (obtained from H. Green) were plated at 2.5×10^5 cells per 35-mm Falcon dish in 2 ml DME containing 10% (v/v) calf serum (KC Biological), penicillin (100 IU ml^{-1}), streptomycin ($100 \mu\text{g ml}^{-1}$) (Gibco) and L-glutamine (0.1 mg ml^{-1}). Cells were grown at 37°C in a 10% CO_2 -90% air atmosphere. After 24 h the medium was removed and replaced with DME containing 10% calf serum, 5% serum or 2% serum. Other dishes received 10% serum plus 10% (w/v) dextran (Sigma, 500,000 molecular weight), 10% (w/v) Ficoll 400 (Pharmacia, 400,000 molecular weight), or 2% (w/v) methylcellulose (Fisher, 15 centipoise). Medium was sterilised by UV radiation. 3 d after the medium change, some dishes received fresh medium. 3 d and 5 d after the first medium change cells were removed by trypsinisation and counted using a haemocytometer. Each value is the average of duplicate dishes. Stock 3T3 cells were maintained as described⁵. Relative viscosity of the medium was determined using a Cannon-Manning semi-microviscometer equilibrated in a water bath at 37°C .

received 10% serum plus 10% dextran (w/v), 10% Ficoll (w/v) or 2% methylcellulose (w/v). After 3 d the cell number was determined, and the final saturation density was found to be sensitive to serum concentration (Table 1). This shows that in the conditions of this experiment the cells were responsive to changes in the effective serum concentration. These cell numbers represented a saturation density, because dishes which received fresh medium at 3 d showed no significant increase in cell number at 5 d (Table 1). Increasing the relative viscosity of the medium by as much as 25-fold by the addition of various polymers had no significant effect on the saturation density. The diffusion coefficient is inversely proportional to the viscosity of the medium, as stated by the Stokes-Einstein relation⁵. If delivery of a growth factor to the cell is diffusion limited, increasing the viscosity would decrease the diffusion coefficient of the factor, and therefore its rate of delivery, in direct proportion to the increase in medium viscosity. The concentration at the cell surface of growth-limiting factors such as those found in serum would be determined by the rate of depletion by the cells and by the rate of replenishment by diffusion from the bulk medium⁶. If cell division ceases at a critical local concentration of growth factors, then this critical concentration would be reached at a lower cell density when the medium viscosity is increased, resulting in a lower saturation density. In medium with 2% methylcellulose, the expected rate of delivery, and therefore the effective concentration at the cell surface of a diffusion-limited substance, would be decreased 25-fold. But as shown in Table 1, only a twofold reduction in the bulk serum concentration produced a significant drop in the saturation density. Because there was no effect of viscosity on saturation density, the saturation density cannot be determined by diffusion-limited delivery of a serum component. A theoretical discussion of the model used in the Stoker experiments suggests that a twofold change in the rate of delivery of a limiting substance would significantly change cell growth⁶. In our experiments, the change in viscosity would alter the rate of delivery of any substance by much more than twofold. That there was no reduction in cell number per unit area even at high medium viscosities therefore argues against a diffusion-limited process determining the saturation density.

When cells were plated at between 15×10^4 and 20×10^4 cells per dish and grown in 10% Ficoll or 10% dextran, the apparent rate of growth was the same as that of cells grown in control medium (Fig. 1). When cells were plated at between 1 and 2×10^4 cells per dish and grown in 10% Ficoll or 10% dextran, after 3–5 d the rate of growth was reduced compared to growth in control medium, and the cells had become obviously vacuolated. The saturation densities of cells in 10% Ficoll and 10% dextran were 65% and 56% of control, respectively (results not shown). These effects of Ficoll and dextran on growth are presumably due to toxicity which is detectable after more than 3 d in culture. Cells plated at 2×10^4 cells per dish and grown in 1% or 2% methylcellulose had a normal appearance, with saturation densities 98% and 88%, respectively, of cells in control medium (Fig. 1). Although these cells grew at a somewhat lower rate than control cells, this difference was small and was not proportional to the relative viscosity of the medium (2.5-fold higher for 1% methylcellulose, 25-fold higher for 2% methylcellulose).

One possible objection to our interpretation of the lack of effect of viscosity on saturation density is that the relative viscosity we measured was in the bulk medium. Thus, the measured viscosity might not reflect the viscosity in the hypothetical boundary layer which could be lower if polymer was excluded near the cell surface. We believe this is very unlikely, first, because the polymers we used

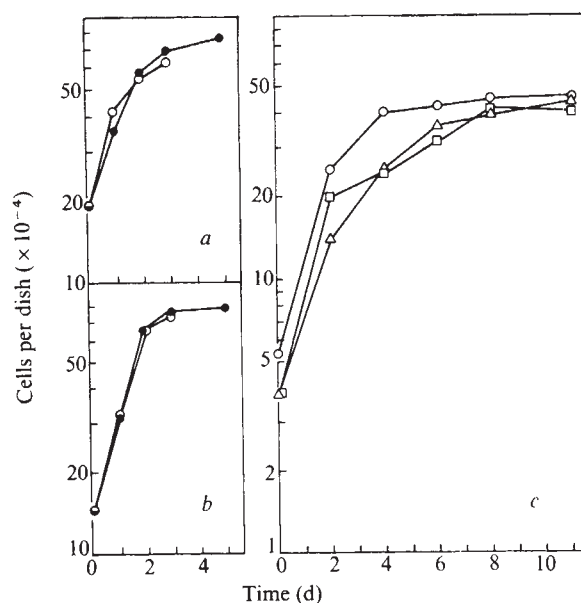


Fig. 1 Effect of viscosity on cell growth. Cells were plated at 22×10^4 (a), 15×10^4 (b) or 2×10^4 (c) cells per 35-mm dish, and treated as in Table 1. a And b, dishes counted on day 5 were fed on day 3. Day 0 is the time at which medium was changed to medium with or without polymer. c, Dishes fed every 3 d. Day 0 is 24 h after changing to medium with or without polymer. Cell number in duplicate dishes was determined as in Table 1. a, 10% Dextran (○); no polymer (●). b, 10% Ficoll (○); no polymer (●). c, 1% methylcellulose (△); 2% methylcellulose (□); no polymer (○).

are not charged, so electrostatic repulsion should not occur, second, the diffusion boundary layer is theoretically of the order of a cell diameter; thus, the extracellular matrix should not exclude the polymers⁶, and finally, because ferritin coupled to concanavalin A, a complex of molecular weight approximately 550,000, is capable of reaching and labelling the cell surface of growing 3T3 cells⁷. The size of this complex approximates the size of the polymers we have used.

Our results are inconsistent with the results expected if saturation density is determined by diffusion-limited availability of a growth-promoting substance. We have recently shown that a surface membrane-enriched fraction will inhibit the rate of DNA synthesis in sparse cultures of 3T3 cells in a manner that mimics the effect of confluent cell density⁸. We believe that the inhibiting effect of membranes is most easily explained by the notion that the membranes provide a cell surface component which interacts directly with a complimentary component on the intact cell, thus providing a negative signal for growth. Taken together, our data support the idea that regulation of cell growth depends on both a negative signal from cell contact and a positive signal determined by the bulk medium concentration of growth-promoting factors such as those found in serum.

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Cytological detection of crossing-over in BUdR substituted meiotic chromosomes using the fluorescent plus Giemsa technique

THE relationship between chiasmata and crossing-over has long been of interest, for if chiasmata are the cytological manifestations of recombination events they represent an easily accessible and reliable source of information on the frequency and distribution of crossing-over throughout the entire genome. Autoradiographic studies on meiotic chromosomes have proved that chiasma formation involves breakage and reunion of homologous, non-sister chromatids^{1–4}. However, the inherent lack of precision in autoradiography has demanded that the stages of meiosis investigated, usually anaphase I or later, are remote from the time of crossing-over, and after homologous chromosome pairs have disjoined. This has precluded a comparison of chiasma distributions with cross-over positions, with one exception. In the grasshopper *Stethophyma grossum*, the proximal localisation of chiasmata at metaphase I has been shown to correspond perfectly with the exchange positions of autoradiographic label at anaphase I (ref. 4).

This report describes the successful application of the fluorescent plus Giemsa (FPG) technique to the meiotic chromosomes of *Locusta migratoria*. The FPG technique allows differentiation of sister chromatids of mitotic chromosomes without autoradiography. After two rounds of replication in the presence of the base analogue 5-bromodeoxyuridine (BUdR) one chromatid of a chromosome has unifilar substitution of its DNA duplex, whereas the other becomes bifilarly substituted with BUdR. The former chromatid stains more intensely than the latter after treatment with the fluorochrome Hoechst 33258 and Giemsa⁵. Differentiation also appears if chromosomes undergo a first replication in the presence of BUdR and a second in its absence⁶. Sister chromatid differential staining obviously has a great potential for re-investigation of the relationship of chiasmata and crossing-over, as it should allow direct comparisons of the positions of

physical exchanges (cross-overs) with the positions of chiasmata in diplotene, diakinesis and metaphase I bivalents. To date only one report has been published of differential staining of meiotic chromosomes, but insufficient differentially stained autosomal bivalents were obtained for analysis of recombination exchanges⁷.

Adult male locusts were injected twice with 50 µl 0.05 M aqueous solution of BUdR with a 5-h interval between the first and second injections. The locusts were kept in an incubator at 30 °C and testes were dissected from animals 9, 10 and 11 d after the start of the experiment and fixed in 1:3 acetic alcohol. Two or three follicles were squashed in 45% acetic acid on each slide. The coverslips were removed after freezing in liquid nitrogen and the slides allowed to dry in air. The differential chromatid staining schedule used was basically that described by Perry and Wolff⁸. Slides were immersed in 0.5 µg ml⁻¹ Hoechst 33258 in phosphate buffer (pH 7.0) for 10 min at room temperature, rinsed once in distilled water and left to age in daylight in a moist chamber for 24 h. After incubation in 2×SSC at 60 °C for 30 min, slides were stained in 5% Giemsa in phosphate buffer (pH 7.0) for 5 min. One of the two locusts sampled on day 10 showed differential sister chromatid staining and all results described below are from this individual. To simplify the task of clarifying the relationship of

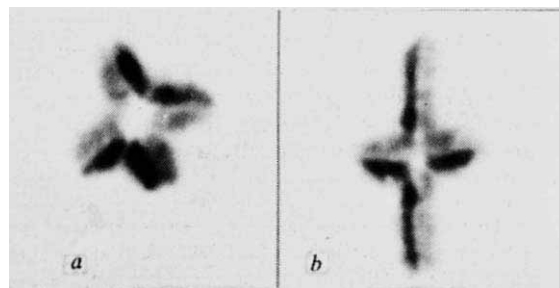


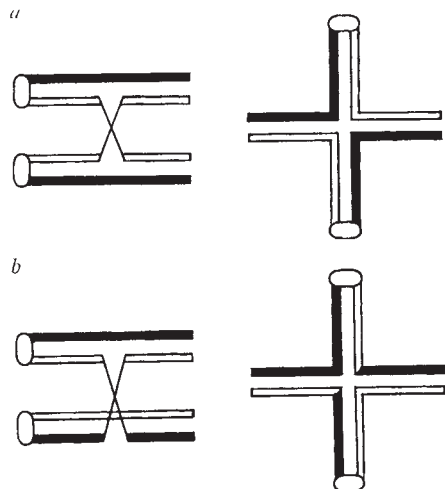
Fig. 2 Differential sister chromatid staining of two diakinesis bivalents. *a*, Type 1 cross-over, no visible exchange generated. This situation is explained diagrammatically in Fig. 1*a*. *b*, Type 2 cross-over, visible exchange of differentially stained chromatid segments shown. The generation of visible exchange is explained in Fig. 1*b*.

chiasmata to cross-overs, only bivalents with a single chiasma were examined in detail.

When homologous chromosomes with differential sister chromatid staining are paired at meiosis, two categories of cross-overs are expected. First, crossing-over may occur between non-sister chromatids which are similarly substituted with BUdR and therefore are similarly stained (type 1). Cross-overs of this type should not generate visible exchanges of differentially stained chromatid material, although they will result in chiasmata (Figs 1*a* and 2*a*). Second, cross-overs may occur between dissimilarly BUdR-substituted, and therefore differentially stained, chromatids (type 2). These cross-overs, in contrast to type 1 exchanges, are expected to produce visible exchanges of light and darkly stained chromatids (henceforward referred to as visible exchanges), as well as producing chiasmata (Figs 1*b* and 2*b*).

A total of 46 monochiasmate bivalents from the single specimen of *L. migratoria* were seen to have adequate differential staining of chromatids for unambiguous analysis of exchanges. Twenty-five of these bivalents lacked visible exchanges of differentially stained chromatids (type 1), whereas the remaining 21 bivalents each contained a visible exchange (type 2). As it is expected that cross-overs will involve any two non-sister chromatids at random, then the numbers of type 1 and type 2 monochiasmate bivalents should be equal. A χ^2 test revealed that the observed numbers of type 1 and type 2 exchanges did not differ significantly from this expectation ($\chi^2_1 = 0.348$, $P > 0.50$). This identification of chiasma-associated visible exchanges is further proof of the formation of

Fig. 1 Expected distribution of light and dark stained chromatid segments after crossing-over. *a*, Cross-over between non-sister chromatids similarly substituted with BUdR does not generate visible exchange of differentially stained chromatid material. *b*, Cross-over between non-sister chromatids dissimilarly substituted with BUdR generates visible exchange.



chiasmata by breakage and reunion of non-sister chromatids, confirming previous autoradiographic demonstrations. Sister chromatid exchanges were also observed in these differentially stained bivalents and in the univalent X chromosome. However, they were relatively infrequent compared with chiasma-associated visible exchanges. A detailed analysis of these is deferred to a further publication.

Careful examination of type 2 exchanges offers an opportunity to determine the importance in this species of the phenomenon known as 'chiasma terminalisation'. It is often suggested that the positions of chiasmata when viewed at late prophase I or at metaphase I do not necessarily reflect the positions of their formation because of their movements towards the telomeric ends of the chromosomes^{8,9}. My results exclude the possibility of chiasma movement in monochiasmate bivalents in *L. migratoria*. In all bivalents with visible exchanges, the positions of exchange coincided with the chiasma as expected if chiasmata retain their original positional correspondence with cross-overs (Fig. 2b). Any degree of chiasma terminalisation would be readily detected by this method since the chiasma would be displaced relative to the visible exchange. It could, however, be argued that the incorporation of BUdR causes localisation of chiasmata at exchange points by arresting terminalisation. To investigate this point, the chiasma distribution pattern at metaphase I in monochiasmate bivalents of the BUdR-treated individual was compared with that of an untreated individual (Table 1). A $2 \times j$ contingency test showed that chiasma distributions in treated and untreated animals did not differ significantly ($\chi^2_2 = 1.446$, $P > 0.30$); that is, there was no evidence that incorporation of BUdR affected chiasma distribution in the way predicted by a postulated arrest of terminalisation.

Table 1 Frequencies of proximal, interstitial and distal chiasmata in 33 monochiasmate bivalents from a BUdR-treated insect and in 50 monochiasmate bivalents from an untreated control insect

	Chiasma position			(Terminal)
	Proximal	Interstitial	Distal	
BUdR-treated	9	5	19	(7)
Untreated control	18	10	22	(12)

Among the 21 monochiasmate type 2 bivalents (that is, those containing a visible exchange at the chiasma), two bivalents were observed to show an anomalous and puzzling type of exchange. In these bivalents the positions of visible exchange points were adjacent to each other instead of being situated diagonally opposite across the chiasma as expected. These anomalous exchange patterns cannot readily be explained by the conventional view of crossing-over occurring between two non-sister chromatids, but they could possibly be accounted for if chiasma formation occasionally occurs by multiple exchanges at one site involving more than two of the chromatids. Further investigations are under way to determine the frequency of such configurations and thus their possible significance and importance.

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Sex difference in Lewis rats in the incidence of recurrent experimental allergic encephalomyelitis

EXPERIMENTAL allergic encephalomyelitis (EAE) can readily be produced in several animal species by the injection of central nervous system (CNS) tissue or the basic protein of CNS myelin with or without adjuvants¹. Although EAE is usually a non-recurring and self-limiting process, recurrent EAE has been reported to occur spontaneously in rats² and guinea pigs³ and also in specific conditions in the rat⁴. Investigations on rats have previously used female rats^{2,4}. I report here a striking difference between male and female Lewis rats in the incidence of spontaneous recurrent EAE.

Table 1 shows that the mean day of onset and the severity of the disease were similar in both sexes. Eighteen (45%) of the females, however, had a recurrent episode which was as severe as the first attack. All the males had one episode with subsequent recovery at about 20 d post-immunisation, with the exception of three, which were found dead. Quantitative assessment of perivascular cellular infiltrates revealed no difference in severity between recurrent and monophasic EAE in rats. It is possible that differences were not observed at 45 d, when the experiment was terminated, because EAE lesions in the CNS were resolving.

Clinical relapse in the females occurred, on average, 6 d after the clinical score had returned to zero. McFarlin *et al.*² considered the possibility that relapses were caused by a second immune response of a different type, or one directed to a different determinant from that which triggered the initial attack. If this were so, the same incidence of relapse would be expected in both sexes. Levine and Sowinski⁴ postulated that spontaneous relapse in the rat was due to a reactivation of residual or partially unprocessed antigen. They proposed that nonspecific stress with increased secretion of adrenocortical hormones occurs in EAE. The corticosteroids exert anti-inflammatory and immunosuppressive effects which promote recovery and the animal returns to its normal endocrine and immunological status. It is during this time, when adrenal hyperactivity subsides, that residual or partially processed antigen may be used to initiate another episode of EAE. These postulates were supported when Levine and Sowinski showed that adrenalectomy soon after an EAE attack caused a marked increase in the relapse rate.

Regulation of adrenal glucocorticoid secretion is achieved largely by negative feedback control of corticotropin-releasing factor from the hypothalamus and by direct effects on the adenohipophysis⁵. Adrenal responsiveness to stressful stimuli, however, involves an 'open loop' mechanism, wherein regulation by negative feedback becomes temporarily ineffectual⁶. Changes in corticosterone secretion associated with reproductive function must ultimately be integrated within the framework of these control systems.

One explanation for the sex difference in recurrent EAE could be the polyoestrous cycle in the female rat. Experimental manipulations which stimulate adrenal function could alter the rate of progesterone and corticosterone output in ovarian and adrenal effluent during the oestrous cycle. These alterations may have a direct effect on the hyperactivity of the adrenal glands, thereby creating a premature 'switch-off', as is observed in adrenalectomised rats. It is interesting to note that the period between remission and relapse in spontaneous and adrenalectomy-induced recurrent EAE averaged 6 d, and that the oestrous cycle of the rat is 4 d. Further evidence for hormonal effects on the course of EAE comes from studies on EAE in pregnant rats and guinea pigs⁷.

Investigations are now being pursued using castrated oestrogen-treated male rats and ovariectomised females in an attempt to elucidate the incidence of relapses. This may yield some in-

Table 1 Induction and clinical observations of recurrent EAE in Lewis rats

Sex	No. of rats	Episode 1 Mean day of onset	Mean maximal* clinical score	No. of rats	Episode 2 Mean day of onset	Mean maximal clinical score
Female	40	11.8	3.7	18	22.6	3.4
Male	60	12.2	3.9	0	—	—

Adult Lewis rats were injected intracutaneously into the dorsum of each hind foot (0.1 ml per foot) with fresh guinea pig spinal cord homogenised in saline (1:1 w/v) and emulsified with an equal volume of Freund's complete adjuvant containing 10 mgml⁻¹ of *Mycobacterium tuberculosis* (Difco H37Ra).

*Scores for clinical symptoms were graded as follows: 1, transient weight loss and limp tail; 2, hind limb weakness; 3, moderate paraparesis and/or incontinence; 4, severe paraparesis; 5, death.

formation regarding the mechanism of relapse in EAE and possibly human demyelinating diseases.

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Electron microscopy of the organism of Legionnaires' disease

LEGIONNAIRES' disease, an acute lobar pneumonia with a considerable fatality rate, gained its name from the explosive outbreak which followed an American Legion convention in Philadelphia in July 1976 (ref. 1). Since then, some smaller outbreaks and an increasing number of sporadic cases have been recognised in the USA². There have also been reports of cases in the UK, from Glasgow, mainly associated with holiday travel^{3,4}, and, as an indigenous occurrence, from Nottingham^{5,6} and London⁷. The causative organism, a small Gram-negative bacillus, has been cultured with some difficulty, its size being 0.3–0.5 μm in width and 2–3 μm in length, though longer forms up to 20 μm are not uncommon⁸. We present here electron micrographs showing the organism in post mortem lung tissue.

The lung tissue came from a patient who died on 19 June, 1976 with extensive lobar pneumonia. The tissue had been shown (unpublished data) to harbour the organism of Legionnaires' disease by specific fluorescent antibody and silver impregnation staining⁹. Electron microscopy of the lung tissue was carried out by negative staining after emulsi-

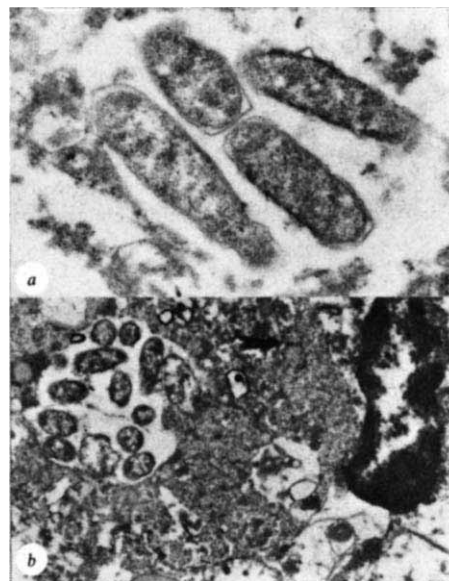


Fig. 2 Electron micrographs of thin section lung tissue with the organism *a* in the extracellular spaces, showing ribosomes and loose outer membrane ($\times 60,000$); *b*, as an inclusion within the cell cytoplasm ($\times 25,000$). Tissues were initially formalin fixed, rinsed thoroughly in cacodylate buffer pH 7.2, fixed in 3% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated in ethanol and embedded in a mixture of Epon 812 and Araldite CY212. These sections were cut on an LKB ultra-microtome, stained with uranyl acetate and lead citrate and examined in a Cornith 500 electron microscope.

fication and by thin sectioning after embedding. In the negatively stained material short cocco-bacilli were present, about 0.6 μm in width and 1–2 μm in length, with non-parallel sides and tapered ends. The bacillary surfaces were smooth; vacuole-like bodies were evident and occasional bacilli showed a loose outer membrane (Fig. 1a). Some organisms had an irregular convoluted surface, were curved and contained no vacuoles (Fig. 1b); others were seen in the process of dividing by binary fission. Long forms of the

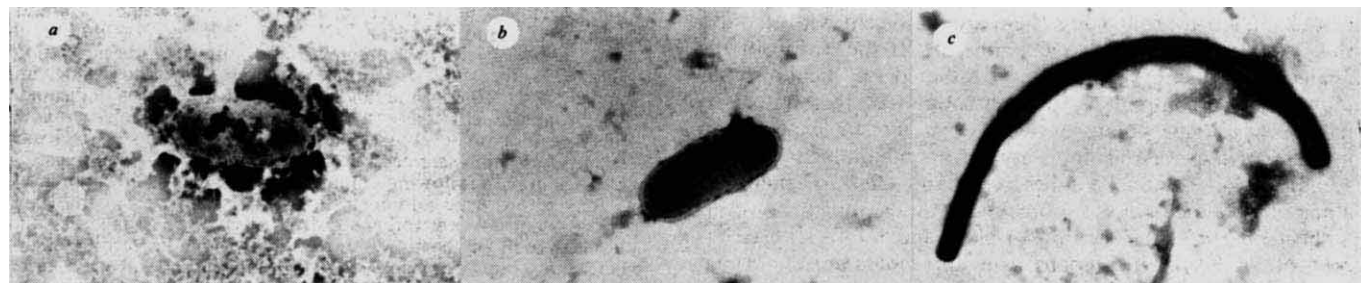


Fig. 1 Electron micrographs of the Legionnaires' disease organism. Emulsified lung tissue in distilled water was washed, diluted, negatively stained with 1% phosphotungstic acid at pH 6.5, and examined in an AEI Cornith 500 electron microscope. *a*, The smooth coccobacillary form with vacuoles and outer membrane ($\times 48,000$); *b*, the type showing convoluted surface detail ($\times 48,000$); *c*, a highly curved long form ($\times 32,000$).

organism were rarely observed (Fig. 1c). In thin sections the appearance of the organisms was consistent with the known properties of Gram-negative bacilli. They measured 0.3–0.5 μm in diameter, with a limiting cell envelope comprised of a double membrane of variable width, the outer lamella of which seemed to be loose and undulating. At its narrowest, the double membrane was approximately 25 nm wide. The bacterial contents were granular due to the presence of ribosomes; some organisms contained vacuoles (Fig. 2a), and fine thread-like structures in the cytoplasm. These organisms were found in the extracellular spaces of the lung tissues but were also distributed singly and in groups in vacuoles in the cytoplasm of degenerating lung cells (Fig. 2b). No bacilli were observed within the nuclei. Whether the organisms in the extracellular spaces had replicated there or were indeed from infected tissues is not known, though from *in vitro* studies it is possible that the coccobacillary form of the organism develops intracellularly and the long form extracellularly.

This brief report on one case of Legionnaires' disease does not presume to reflect the complete morphology of the organism *in vivo*, but the intracytoplasmic development and unusual structure by negative staining electron microscopy are of interest.

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The narcotic antagonist naloxone enhances clinical pain

ENDOGENOUS opiate-like substances (endorphins) have been isolated from the brain in a variety of species, including man^{1,2}. Furthermore, there is recent evidence that the brain possesses an analgesia-producing system. The distribution of endorphins is consistent and overlaps significantly with central nervous system sites such as the midbrain periaqueductal grey which produce analgesia when activated by electrical stimulation or opiate microinjection^{3–5}. This raises the possibility that the analgesia is mediated by endorphin release. At present very little is known about the factors which trigger endorphin release. One obvious possibility is that the pain suppression system is activated by noxious stimuli. In fact, it has been observed that noxious stimuli delivered at one region of the body reduce the reported intensity of a distant noxious stimulus⁶. Furthermore, studies of the component neurones of this analgesia-producing system are consistent with the idea that it is activated by noxious stimuli^{7,8}. If the action of the analgesia-producing system is mediated by endogenous morphine-like compounds, then disruption of this system by naloxone, a pure opiate antagonist, would be expected to increase the perceived intensity of a maintained noxious stimulus. Some animal studies report no effect of naloxone alone on baseline pain thresholds^{9,10}; in other studies, however, a shortening of latencies on hot-plate and tail-flick tests has been observed^{11,12}. In humans, pain thresholds are unaffected by naloxone alone^{13,14}. However, reversal of analgesia produced by electrical stimulation^{15,16} or by acupuncture¹⁷ has been reported. In 1965 Lasagna commented on the "curious hyperalgesic effect" of naloxone in patients with postoperative pain¹⁸. These observations suggested that clinical pain might

provide a more appropriate model to study the action of endorphins. We demonstrate here that following the extraction of impacted wisdom teeth, naloxone causes a significantly greater increase in reported pain intensity than placebo, an observation consistent with the suggested participation of endorphins in an intrinsic pain suppression system.

All but 3 of the 26 subjects were private patients in the Department of Oral Surgery of the University of California, San Francisco. Patients with spontaneous pain before surgery were excluded. The mean initial pain level was zero in all patients. The group consisted of 15 males and 11 females, with a mean age of 22 yr (range 16–32 yr). Consent to act as a subject was obtained on a form following the guidelines of the campus committee on human experimentation. Patients were informed of the time course of the experiment (Fig. 1) and were told that they would receive one or more of the following three drugs: morphine, a "standard medication in the treatment of pain"; placebo, a class of substances which has been shown to "partially relieve postsurgical pain in more than one-third of patients", and naloxone, a drug which "may be somewhat less effective than placebo in terms of pain relief, and theoretically may increase pain".

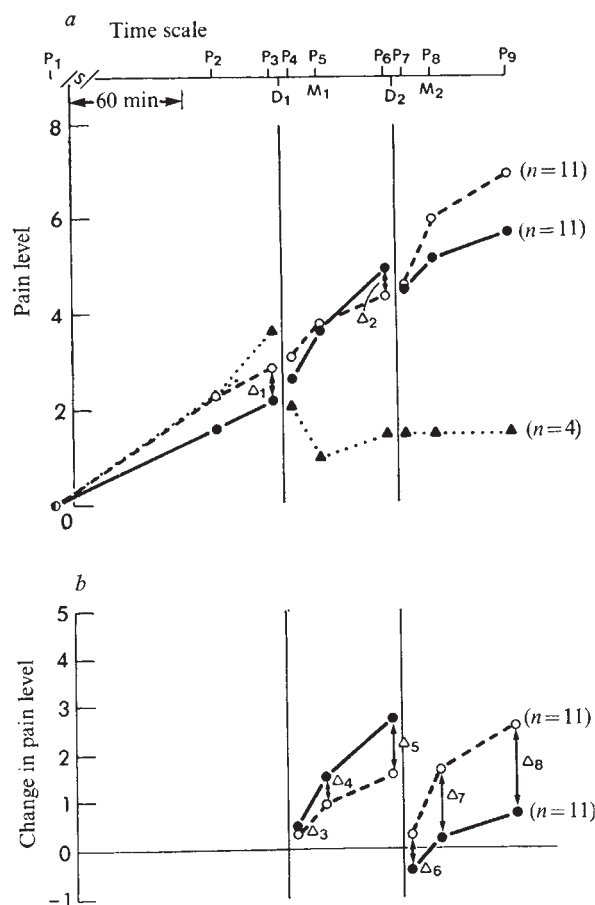


Fig. 1a, Time scale for all parameters is at the top. Anaesthesia and surgery (S), and times of pain level (P_n , $n=1-9$) and mood scale (M_n , $n=1,2$) measurements and of drug administration (D_1 , D_2) are shown. Pain levels were reported using an uncalibrated 10-cm line and plotted at the times indicated. All groups except those treated with morphine show a steady increase in pain levels (see text). Naloxone seems to increase the rate of pain increment for both N→P and P→N groups. b, Change in pain following administration of drugs. The pain level 5 min before drug administration is subtracted from the level at 5, 20 and 60 min following drug to give the change in pain level at each of the times shown. The Δ 's indicate differences between groups receiving placebo and naloxone at each time (see Table 1, $n=1-8$). Naloxone produces a greater increase in reported pain levels than placebo. The significance of these differences is given in Table 1. A profile of mood scale²⁵ was obtained at times indicated by M_n . Analysis of these scales showed no correlation with placebo, naloxone or morphine. ●, N→P; ○, P→N; ▲, M→P.

A random double-blind cross-over design was used. The design and time course is shown in Fig. 1. One patient group ($P \rightarrow N$) received placebo (that is, naloxone vehicle) as the first drug (D_1) and naloxone (9 mg) as the second drug (D_2). Another group ($N \rightarrow P$) received the same drugs in the opposite sequence. A third group ($P \rightarrow M$) received placebo followed by 7.5 mg of morphine sulphate. All drugs (or vehicle) were administered as a bolus of identical volume (3 ml) through an intravenous catheter in full view of the patient. Patients were not told that the substance administered was an effective drug. No immediate effect of placebo, naloxone or morphine which could have enabled a patient to differentiate these drugs was reported.

A standardised surgical procedure for the removal of impacted molars was used. From two to four teeth were removed, including at least one impacted mandibular third molar. All patients were premedicated with 10–20 mg of diazepam. During surgery, nitrous oxide was used in concentrations of 15%–40%. In addition, local anaesthesia was produced with 3.0% mepivacaine without vasoconstrictor. This solution has an effective duration of 45–75 min. Zero time for each patient began with the onset of local anaesthesia. Immediately following completion of surgery, N_2O was flushed with 100% oxygen for 10 min. The timing of pain level measurements and drug administration are shown in Fig. 1.

Pain was evaluated using a visual analogue scale consisting of a 10-cm horizontal line on a card with the words 'no pain' on the left end and 'worst pain ever' on the right end. At prescribed intervals (Fig. 1) patients were given a card and asked to mark the horizontal line at a point representing their pain. The scale was later rated to the nearest millimetre, and that score recorded. The validity of this visual analogue scale for pain measurement has been verified by other investigators^{19,20}, and, in this study, correlated better than 95% with verbal reports of change in pain levels.

Figure 1a plots the averaged pain scale ratings for the three groups, $P \rightarrow N$, $N \rightarrow P$ and $P \rightarrow M$. In Fig. 1b, the effects of naloxone and placebo are compared for the first and second hours of the study. The pain ratings varied among the patients in each experimental group and differences between the pain levels for naloxone and placebo groups were not significant at any time point. However, if the change in pain rating at 5, 10 and 60 min following drug administration is compared to the rating 5 min before administration, significant differences occur between naloxone and placebo (Fig. 1b, Table 1).

The probabilities of the pairs of mean values being identical (Student's *t*-test) are shown in Table 1. When the groups receiving naloxone and placebo as D_1 are compared, the naloxone group shows a greater increase in pain. This difference reaches significance at 60 min. When the effects of these drugs as D_2 are compared, the difference reaches significance at 20 min.

These results indicate that the surgical procedure described is associated with the release of an endogenous morphine-like compound. Although naloxone reversal of N_2O analgesia has been reported in rats²¹, in this study pain estimates were made no sooner than 90 min after cessation of N_2O . The possibility that diazepam may produce naloxone-reversible analgesia has not been directly examined but seems unlikely, as it has not been shown to be a parenteral analgesic²² or to have opiate-like properties²³. Furthermore, the difference between naloxone and placebo groups is greater in the hour following the second experimental drug (D_2) when the previously administered diazepam and mepivacaine levels are lower. The effect of naloxone on pain was maximal at one hour following intravenous injection, although the onset of action seems to be much earlier (Fig. 1b). This time course of action is consistent with the metabolism of naloxone²² and recent work showing naloxone reversibility of stimulation-produced analgesia²⁴.

The experiment provides evidence that an endogenous morphine-like factor is released in a clinical situation and is

Table 1 Comparison of probability

	P
Δ_1	NS
Δ_2	NS
Δ_3	NS
Δ_4	NS
Δ_5	< 0.05
Δ_6	NS
Δ_7	< 0.05
Δ_8	< 0.01

NS, not significant.

consistent with the observations that in man, analgesia produced by diencephalic stimulation^{15,16} or acupuncture¹⁷ is reversed by naloxone. These observations further indicate that pain is an important activating factor of the endorphin-mediated analgesia system. It may be that the prolonged duration and stress associated with clinical pain as opposed to experimental pain make it particularly effective for activating the endogenous analgesia system.

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Insulin receptors are widely distributed in the central nervous system of the rat

WHILE insulin can affect the function of the central nervous system (CNS) by producing hypoglycaemia, there is substantial evidence suggesting that insulin can also act directly on cells of the CNS to modify their function^{1–4}. In a study of the phylogeny of the insulin receptor, Posner⁵ reported specific binding of ¹²⁵I-insulin to membrane preparations from whole brain of rat, monkey and pigeon. Specific receptors for insulin have been detected in the hypothalamus of monkeys but not in the cerebral cortex or

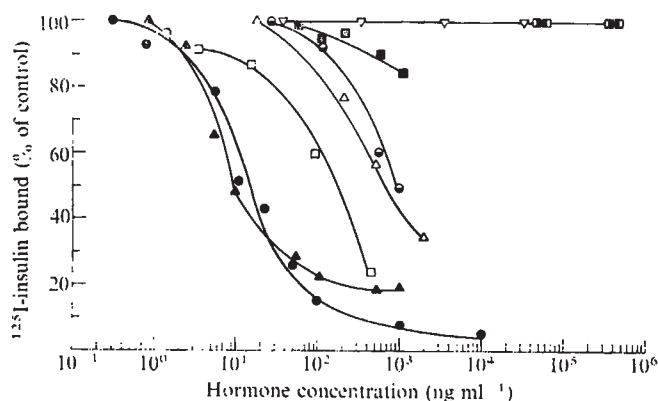


Fig. 1 ^{125}I -insulin binding to cerebral cortex. Cerebral cortex of adult Sprague-Dawley rats (250–300 g) was macroscopically dissected, frozen on dry ice, homogenised at 4°C in an all-glass homogeniser in 40 volumes of 0.001 M NaHCO_3 , and centrifuged for 10 min at 600 g . The supernatants were centrifuged for 30 min at $20,000\text{ g}$; the pellets were washed once with 0.001 M NaHCO_3 and suspended in the incubation buffer (100 mM HEPES , 120 mM NaCl , 1.2 mM MgSO_4 , 2.5 mM KCl , $15\text{ mM NaC}_2\text{H}_3\text{O}_2$, 10 mM glucose , 1 mM EDTA). Porcine ^{125}I -insulin ($130\text{--}180\text{ }\mu\text{Ci }\mu\text{g}^{-1}$) and unlabeled peptides were dissolved in the same buffer except that bovine serum albumin (BSA) was added at a final concentration of 13 mg ml^{-1} . $50\text{ }\mu\text{l}$ of $20,000\text{ g}$ pellet, $50\text{ }\mu\text{l}$ of ^{125}I -insulin (0.3 ng ml^{-1}) and $50\text{ }\mu\text{l}$ of porcine insulin ($\bullet$), chicken insulin ($\blacktriangle$), fish insulin ($\square$), desalanine-desasparagine insulin (DAA) ($\triangle$), proinsulin ($\odot$), MSA, multiplication stimulating activity, an insulin-like peptide purified from the culture medium of a rat liver cell line ($\blacksquare$), hGH (∇), A-chain ($\blacksquare$) and B-chain ($\blacksquare$) of insulin were incubated for 90 min at 15°C , following which the tubes were centrifuged in a Beckman microfuge. The supernatants were removed and used to measure degradation of ^{125}I -insulin (which, estimated by precipitation with trichloroacetic acid, ranged between 4 and 10%). The radioactivity in the membrane pellet was counted ('total binding'). The binding of ^{125}I -insulin in the presence of $100\text{ }\mu\text{g per ml}$ of unlabeled porcine insulin was considered to be 'non-specific binding' and was subtracted. The specific binding (total binding minus nonspecific binding) was expressed as % of ^{125}I -insulin specifically bound in the absence of unlabeled insulin.

thalamus⁶; other regions of the CNS were not studied. We report here the presence of substantial concentrations of insulin receptors in discrete regions of the CNS of the rat. We detected specific binding of insulin in every area

studied, although it differed by as much as five- to 10-fold among regions. The insulin receptor of the cerebral cortex was most extensively characterised, and by all criteria it was indistinguishable from the insulin receptor on classical target tissues (liver, muscle and fat) as well as other cells of humans, rodents^{7–10}, and other mammals and non-mammalian vertebrates.

^{125}I -insulin bound to cellular components prepared from cerebral cortex (Fig. 1). High concentrations of unlabeled insulin ($100\text{ }\mu\text{g ml}^{-1}$) reduced the binding of ^{125}I -insulin to about 30% of the control level (this residual binding is referred to as nonspecific binding). Physiological concentrations of unlabeled insulin ($1\text{--}5\text{ ng ml}^{-1}$) competed with ^{125}I -labelled insulin for binding, and half maximal displacement of labelled insulin was seen at insulin concentrations of about 15 ng ml^{-1} . Six insulin-like peptides, differing over a several hundredfold range in their insulin-like biological potencies, all competed with the labelled insulin for binding to receptors of the cerebral cortex, and in each case the effectiveness of the insulin-like peptide in competing for insulin binding correlated with the biological potency of these preparations. Isolated A-chain and B-chain of the insulin, which lack the biological properties of insulin, were without effect on the binding of ^{125}I -insulin to the receptor, as were unrelated hormones such as growth hormone (hGH).

In addition to binding to specific insulin receptors which mediate the metabolic responses of insulin, insulin also binds to specific receptors for somatomedins and other insulin-like growth factors. However, when insulin binds to the growth factor receptors, the binding is of substantially lower affinity, and unlabeled peptides have a distinctly different rank order of potency in competing for binding. Thus the binding to cerebral cortex that we have detected is typical of binding to insulin receptors responsible *in vivo* and *in vitro* for mediating metabolic responses to insulin. In addition to having characteristic affinity and specificity for insulin analogues, the receptors of the cerebral cortex have the characteristic temperature dependence and pH dependence of binding; association of ^{125}I -insulin was more rapid at 30°C than at 20°C , and more so at 20°C than at 15°C , but the steady-state level of binding was inversely related to the temperature, and was maximal at pH 8, with a sharp decline on both sides of the pH optimum; this sharp

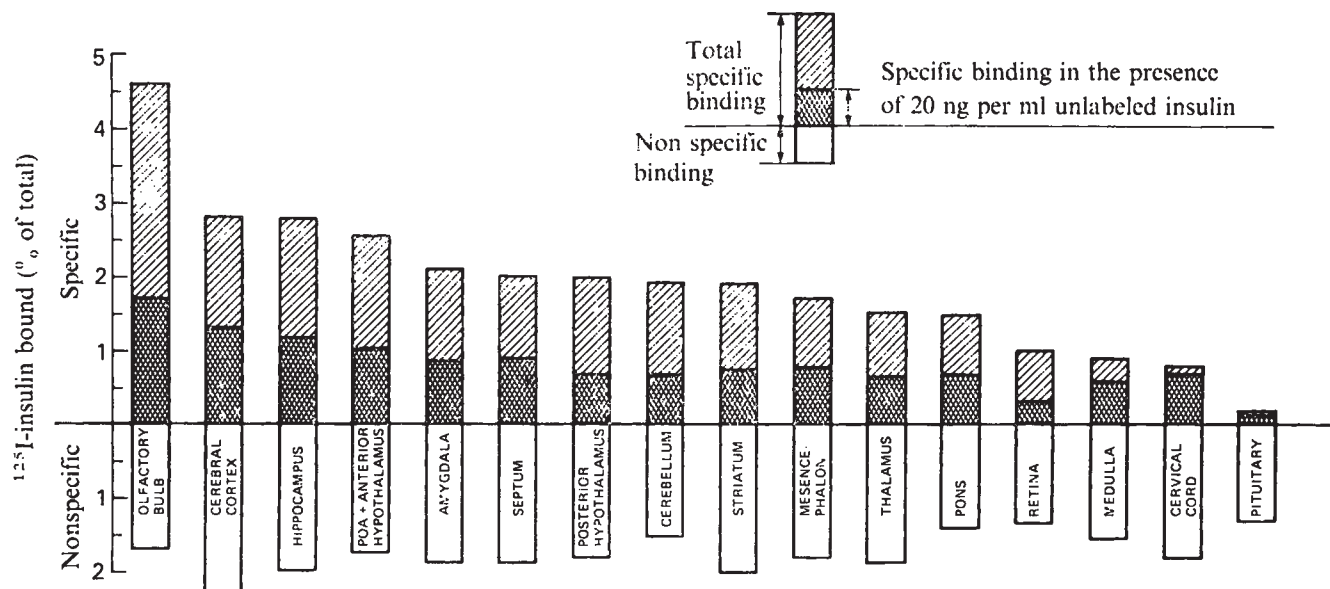


Fig. 2 Regional distribution of the insulin receptor in the CNS of the rat. $20,000\text{ g}$ pellets were prepared and the radioreceptor assay performed as described in the legend of Fig. 1. The specific binding (as % of total radioactivity added) is indicated by the whole hatched bar above the baseline; the double hatched part represents the specific binding in the presence of 20 ng per ml of unlabeled insulin. The empty bar below the baseline represents the nonspecific binding (in the presence of $100\text{ }\mu\text{g per ml}$ of unlabeled insulin). The total, specific and nonspecific binding was adjusted for each region to the protein concentration of $500\text{ }\mu\text{g per ml}$. We showed in a preliminary experiment that specific binding was linear between protein concentrations of $150\text{--}700\text{ }\mu\text{g per ml}$. Degradation of ^{125}I -insulin was determined using the precipitation with trichloroacetic acid (10%) at the end of incubation in every supernatant, and ranged between 5 and 10% .

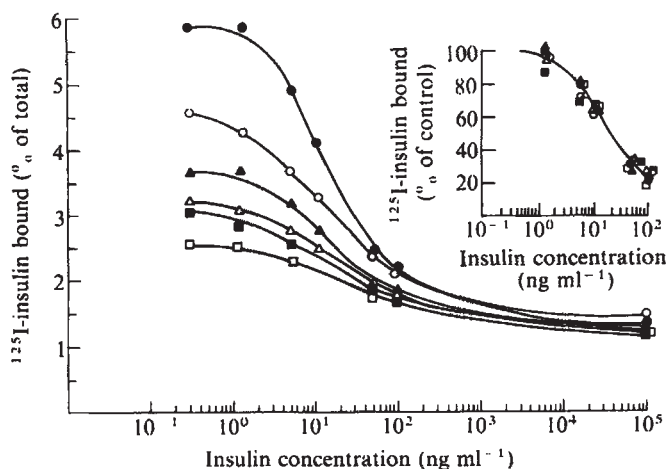


Fig. 3 Binding of ^{125}I -insulin to different regions of the CNS of the rat. 20,000 g pellets from olfactory bulb (●), cerebral cortex (○), preoptic area and anterior hypothalamus (▲), striatum (△), cerebellum (■) and pons (□), prepared as described in the legend of Fig. 1 have been incubated with ^{125}I -insulin (0.3 ng ml^{-1}) for 90 min at 15°C in the absence and the presence of different concentrations of unlabelled porcine insulin. In the main figure the nonspecific binding has not been subtracted; total binding, expressed as % of total counts added, has been adjusted to the protein concentration of $500 \mu\text{g per ml}$ for each region. In the inset the nonspecific binding has been subtracted and the results expressed as % of ^{125}I -insulin specifically bound for each region in the absence of unlabelled insulin.

pH dependence with a pH optimum around 7.8–8.2 is typical of all of the insulin receptors.

Figure 2 shows the distribution of the insulin receptor in the CNS. The specific binding of ^{125}I -insulin (per $500 \mu\text{g}$ of protein) was highest in the olfactory bulb, followed by the cerebral cortex. Other areas rich in insulin receptors were the hippocampus, the preoptic area (POA) plus anterior hypothalamus, amygdala, septum and posterior hypothalamus. Intermediate values were observed with cerebellum, striatum, mesencephalon, thalamus and pons. The lowest values were observed with the pituitary, which is composed of epithelial elements (anterior pituitary) as well as nervous system elements (posterior pituitary) and in the cervical spinal cord, medulla and retina. This characteristic distribution was observed in multiple experiments, with the olfactory bulb uniformly highest. However, in individual experiments, ranking of an area was occasionally shifted one or two places. Whereas specific binding of insulin varied quite markedly among different regions (4.6–0.3% of the total radioactivity), the nonspecific binding was relatively constant at 1–2% of the total radioactivity in all tissues studied.

Figure 3 shows the binding of ^{125}I -insulin as a function of the total insulin concentration for six different regions. Unlabelled insulin competed with labelled insulin in identical fashion, so that the insulin concentration that displaced half of the specific binding is identical for all the tissues. This can be seen even more clearly in the inset of Fig. 3, where each set of data has been expressed as a percentage of its own control.

It is not clear whether the insulin receptors are located on neural tissue, or whether they are on glia or vascular or other supporting tissue. The cellular fraction we used for binding is heterogeneous, containing plasma membranes as well as some myelin sheaths and mitochondria. As this fraction represents only 5% of the total protein of the cell, our results may be prejudiced by preferential recovery of particular cellular components.

Our finding that insulin receptors are widely distributed in the CNS complements reports from several laboratories that insulin can directly affect the CNS function. Debons *et al.*^{11,12} demonstrated a direct action of insulin on the hypothalamic satiety centre. Intravenously administered

insulin can be detected in the cerebrospinal fluid¹³ and in the zona externa of the median eminence¹⁴. Insulin injected into the cerebrospinal fluid or into the carotid artery produces systemic hypoglycaemia^{1–3}.

Not only is the insulin receptor widely distributed throughout the CNS (no region tested was without them) but we found it surprising that regions differed so widely in their receptor content. The finding that the olfactory bulb had the highest binding is especially noteworthy in view of the fact that this region is metabolically very active¹⁵ and its activity can be modified by peripheral injections of insulin¹⁶. It is at present impossible to explain precisely the uneven distribution of insulin receptors in the CNS of the rat although superficially it would seem that receptors are preferentially localised within elements of the limbic system, a complex neuroanatomical system that has been associated with automated activities, including feeding¹⁷.

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Regulation of the glucagon receptor by physiological hyperglucagonaemia

It is well recognised that the first step in the action of polypeptide hormones and catecholamines on target cells is binding of the hormone to a membrane receptor¹. Insulin², catecholamines³ and prolactin⁴ have been shown to regulate the concentration of their own receptors. For example, hyperinsulinaemia due to a variety of causes is accompanied by a decrease in insulin binding⁵, a phenomenon which has been termed 'down regulation'⁶. In contrast, prolactin has been reported to induce its own receptor⁴. In the case of glucagon, the effect of hyperglucagonaemia on the binding of this hormone by target cells has not been established. In starvation, hyperglucagonaemia has been associated with decreased glucagon binding⁷, whereas in uraemia hyperglucagonaemia has been accompanied by an increase in glucagon binding⁸. Since the concentrations of a variety of substrates and hormones are altered in such conditions as starvation and uraemia, the precise role of changes in circulating glucagon in mediating alterations in glucagon binding cannot be delineated from observations in starved or uraemic rats. The present study was consequently undertaken to evaluate the effects of hyperglucagonaemia induced by infusion of physiological amounts of this hormone on glucagon binding in healthy rats. Our findings demonstrate that hyperglucagonaemia lasting 5 h results in a 45% decrease in glucagon binding and a comparable decrease in glucagon-stimulated adenylate cyclase activity. These data thus provide evidence for the 'down regulation' of the glucagon receptor by physiological hyperglucagonaemia.

As shown in Table 1, which contains details of the methods used, the glucagon infusion of rats resulted in a five- to sixfold

increase in plasma glucagon levels. The increments in plasma glucagon achieved in this study are comparable to the glucagon concentrations observed with physiological stimuli to glucagon secretion such as protein ingestion¹⁹ or starvation²⁰, and are similar to those reported in diabetic ketoacidosis²¹ uraemia²², and liver cirrhosis²³. In response to the infusion of glucagon, the plasma glucose concentration increased by 22–24 mg per 100 ml at 20 min and returned towards baseline by 3 h. The magnitude and evanescent nature of the glycaemic response to continuous hyperglucagonaemia is similar to that observed in human²⁴ and dog studies²⁵. In the glucagon-infused rats, plasma insulin levels showed a small ($4.5 \mu\text{U ml}^{-1}$) but statistically insignificant increase.

Following glucagon infusion, specific binding of ^{125}I -glucagon in liver membranes (Table 2) remained unchanged after 1 h, tended to decrease slightly (12%) at 3 h ($P>0.5$), and fell significantly at 5 h to values 45% below control ($P<0.001$). In contrast to the decrease in glucagon binding, specific binding of ^{125}I -hGH and specific binding of ^{125}I -insulin showed no significant change in the glucagon-infused rats compared with control rats ($P>0.5$) (Table 2). These data indicate the specificity of the effect of hyperglucagonaemia on glucagon binding in these animals.

Since activation of adenylate cyclase is the next well recognised step in the interaction between glucagon and its target organs, we examined basal and stimulated adenylate cyclase in liver membranes from these animals (Table 2). Basal adenylate cyclase in liver membranes remained unchanged in all three groups of glucagon-infused rats, whereas glucagon-stimulated adenylate cyclase was reduced by 45% in the rats receiving the 5-h infusion of glucagon ($P<0.001$). In contrast to glucagon-stimulated adenylate cyclase activity, sodium fluoride-stimulated adenylate cyclase was identical in the 5 h glucagon-infused rats and the saline controls (Table 2).

Figure 1 shows the effects of varying concentrations of glucagon on the activation of adenylate cyclase and glucagon binding in liver membranes obtained after 5 h of saline or glucagon infusion. In the saline- as well as glucagon-infused rats, saturation

of glucagon-binding sites and activation of adenylate cyclase occurred in the range of 50–75 nM of glucagon. In glucagon-infused rats the amount of glucagon bound at saturation (2.45 ± 0.06 pmol per mg protein) was significantly lower than in saline-infused rats (4.2 ± 0.09 pmol per mg protein) ($P<0.001$). Furthermore, adenylate cyclase activation showed a similar decrease in liver membranes from glucagon-infused rats at all concentrations of glucagon studied. The concentration of glucagon giving half-maximal binding or activation of adenylate cyclase was 3–4 nM in both groups of animals (Fig. 1). Scatchard analysis²⁶ of glucagon-binding data revealed that the binding capacity of glucagon-infused (5 h) rats was 45% lower than that of saline controls ($P<0.001$), whereas the binding affinity was similar in both groups of animals. These data indicate that the decrease in glucagon binding and glucagon-stimulated adenylate cyclase in glucagon-infused rats was due to a decrease in the number of glucagon receptors rather than a consequence of a change in receptor affinity.

The liver membranes used in this study were also characterised in terms of protein yield and marker enzyme activities. The yield of membrane protein was identical in saline- (1.64 ± 0.09 mg per g wet liver weight) and glucagon-infused rats (1.68 ± 0.08). 5'-Nucleotidase activity was also identical in the control (56.3 ± 4.1 μmol 5'-AMP hydrolysed per mg membrane protein per h) and experimental group (51.0 ± 2.4). More importantly, purification of 5'-nucleotidase in the liver plasma membranes compared with the whole homogenate was similar in both groups (17–19-fold). Furthermore, specific activity of the microsomal enzyme, glucose-6-phosphatase, in liver membranes was 35–40% less than that of the whole homogenate in both groups of animals, indicating minimal contamination of these membranes. These data indicate that the changes in glucagon binding reported above are unlikely to be due to a systematic effect of the glucagon infusion on the membrane purification procedure used in this study. Examination of glucagon degradation activity (percentage degradation of ^{125}I -glucagon) in liver membranes showed no significant differences between 5 h saline- and 5 h glucagon-infused rats ($25.0 \pm 1.2\%$ compared with $24.2 \pm 1.6\%$ using TCA precipitation; $36.4 \pm 1.9\%$

Table 1 Plasma glucose, glucagon and insulin levels in saline- and glucagon-infused rats*

Time	Infusion:	60-min infusion (n = 10)			180-min infusion (n = 10)			300-min infusion (n = 12)		
		0	20 min	60 min	0	20 min	180 min	0	20 min	300 min
Plasma glucose (mg 100 ml ⁻¹)	Saline	98 ± 5	98 ± 6	102 ± 6	100 ± 6	104 ± 8	103 ± 8	96 ± 6	100 ± 6	104 ± 8
	Glucagon	96 ± 6	118 ± 4†	112 ± 5†	98 ± 6	122 ± 8†	106 ± 7	100 ± 6	124 ± 6†	104 ± 7
Plasma glucagon (pg ml ⁻¹)	Saline	50 ± 5	—	60 ± 4	52 ± 4	—	58 ± 6	60 ± 4	—	68 ± 8
	Glucagon	54 ± 4	—	286 ± 18‡	60 ± 4	—	290 ± 16‡	56 ± 6	—	294 ± 18‡
Plasma insulin ($\mu\text{U ml}^{-1}$)	Saline	16 ± 3	—	17 ± 4	15 ± 4	—	15 ± 4	16 ± 3	—	18 ± 4
	Glucagon	18 ± 4	—	22 ± 4	16 ± 3	—	20 ± 3	18 ± 4	—	23 ± 3

*Data are presented as mean ± s.e.m.

†Significantly different from basal (0 time) concentrations. $P < 0.05$.

‡Significantly different from basal (0 time) concentrations. $P < 0.001$.

Adult male Sprague-Dawley rats weighing 250–300 g and maintained on standard Purine Chow were used in all experiments. Rats were anaesthetised with pentobarbital 45 mg per kg body weight intraperitoneally, and were studied in groups of four animals (two control and two experimental) using heat lamps to prevent hypothermia during prolonged infusions. Animals were starved for 6–8 h before initiation of infusion by way of an indwelling polyethylene catheter placed in a jugular vein. The experimental group received an infusion of crystalline glucagon (Eli Lilly, Indianapolis, Indiana) dissolved in 0.9% saline in a dose of $3 \text{ ng per kg}^{-1} \text{ min}^{-1}$ for either 1, 3 or 5 h. Control rats received infusions of saline for similar time periods. The infusion rate in both groups of animals was maintained at 2 ml h^{-1} using a constant infusion pump (Harvard Apparatus, Millis, Mass. Model 2681). Arterial blood samples (1.5–2 ml) were collected through the carotid artery at the beginning and at the end of each infusion period for the measurement of plasma glucose, insulin and glucagon. In addition, a 0.2-ml blood sample was drawn at 20 min after the initiation of each infusion for the measurement of plasma glucose. At the end of each infusion period the liver was quickly removed and quick-frozen in liquid nitrogen. Plasma glucose was measured using the glucose oxidase method on a Beckman glucose analyser. Plasma immunoreactive insulin and glucagon (using Unger antibody 30K) were determined by previously reported methods⁹.

Partially purified liver plasma membranes were prepared using the method of Neville¹⁰ as described previously⁸. The preparation sequence was rigidly followed for membranes from all groups of rats. The specific activity of the plasma membrane marker enzyme, 5'-nucleotidase, was measured using the method of Avruch and Wallach¹¹; the microsomal marker enzyme, glucose-6-phosphatase was measured using the method of Swanson¹². Membrane protein was estimated using the method of Lowry *et al.*¹³. ^{125}I -labelled insulin⁹, glucagon⁸ and human growth hormone¹⁴ (hGH) were prepared using a modification of the chloramine-T method¹⁵. The specific activities of the ^{125}I -labelled hormones were between 150–200 $\mu\text{Ci } \mu\text{g}^{-1}$ and trichloroacetic acid (TCA) precipitability of all labelled hormones was $>95\%$.

The binding of ^{125}I -glucagon to hepatic plasma membranes was carried out according to the method of Rodbell¹⁶ as described previously⁸. ^{125}I -insulin binding studies were carried out using the method of Kahn *et al.*¹⁷ as described previously⁸. In the hGH binding studies, monomeric human growth hormone (little GH or LGH) was used¹⁴. Adenylate cyclase activity in liver membranes was measured according to the procedure of Steiner *et al.*¹⁸ as described previously⁸. Stimulation of adenylate cyclase was measured by the addition of $2 \mu\text{M}$ glucagon and 15 mM NaF (ref. 8). Glucagon degradation was determined by TCA precipitation, by binding to fresh liver membranes and by adenylate cyclase activation as described in detail previously⁸.

Table 2 Influence of hyperglucagonaemia on specific binding of glucagon, insulin and growth hormone, and on adenylate cyclase activity in liver membranes*

Time	60 min (n = 10)	Saline infusion 180 min (n = 10)	300 min (n = 12)	60 min (n = 10)	Glucagon infusion 180 min (n = 10)	300 min (n = 12)
Specific binding (%)†						
^{125}I -glucagon	12.8 ± 0.6	12.2 ± 0.6	12.2 ± 0.7	12.1 ± 0.7	10.8 ± 0.8	7.0 ± 0.3‡
^{125}I -insulin	—	—	22.6 ± 1.6	—	—	20.2 ± 1.4
^{125}I -hGH	—	—	28.4 ± 1.8	—	—	29.2 ± 2.0
Adenylate cyclase activity (nmol cyclic AMP per mg protein per 10 min)						
Basal	0.42 ± 0.03	0.43 ± 0.04	0.44 ± 0.05	0.43 ± 0.04	0.42 ± 0.03	0.40 ± 0.04
Glucagon (2 µM)	4.82 ± 0.21	4.66 ± 0.24	4.70 ± 0.30	4.48 ± 0.30	4.01 ± 0.34	2.72 ± 0.18‡
NaF (15 mM)	3.10 ± 0.20	3.12 ± 0.18	3.02 ± 0.16	2.98 ± 0.16	3.00 ± 0.21	3.12 ± 0.14

*Data are presented as mean ± s.e.m.

†The specific binding for each hormone was calculated by subtracting nonspecific binding (labelled hormone bound in the presence of 1,000 ng ml⁻¹ unlabelled hormone) from the total binding (labelled hormone bound in the absence of unlabelled hormone). Data are presented as mean ± s.e.m. for 0.2 mg membrane protein per ml incubation medium.‡Significantly different from saline-infused group ($P < 0.001$).

compared with $34.6 \pm 2.0\%$ as determined by binding to liver membranes; and $38.4 \pm 1.6\%$ compared with $37.6 \pm 1.8\%$ as determined by adenylate cyclase activation).

The current data demonstrating decreased glucagon binding in glucagon-infused rats provide evidence for the regulation of the glucagon receptor by physiological hyperglucagonaemia. Compared with saline controls or rats receiving 3-h infusions of glucagon, glucagon binding fell by 45% in rats infused with glucagon for 5 h. This decrease in glucagon receptors is unlikely to be due to an artefactual masking of glucagon receptors by residual membrane-bound glucagon for several reasons: (1) basal adenylate cyclase in glucagon-treated animals would be expected to be elevated were residual membrane-bound glucagon present; (2) residual membrane-bound glucagon would be expected to manifest itself in the glucagon binding curve as a reduction in hormone binding at low hormone concentration, which was not

observed in this study; (3) glucagon binding did not decrease after 1 h of glucagon infusion at which time *in vivo* receptor occupancy would be the same as at 5 h.

In addition to the decrease in glucagon binding, the current observation that glucagon-stimulated adenylate cyclase activity is similarly reduced suggests that altered glucagon binding may have a regulatory role in diminishing the effects of physiological increments of this hormone on target cells. In this regard, Plas and Nunez²⁷ reported the development of refractoriness to glucagon stimulation of glycogenolysis in cultured foetal hepatocytes after previous exposure to high concentrations (10 nM) of this hormone. This refractoriness to glucagon was shown to be related to decreased cyclic AMP production. Similarly, DeRubertis and Craven²⁸, using pharmacological infusions of glucagon (250 ng kg⁻¹ min⁻¹) reported a decrease in glucagon-stimulated but not in sodium fluoride-stimulated adenylate cyclase activity in rat liver. The present data indicate that a similar decrease in responsiveness of the cyclic AMP system to glucagon accompanies physiological hyperglucagonaemia and is mediated, at least in part, by a reduction in glucagon binding.

It should be noted that the decrease in glucagon binding did not occur until after 5 h of continuous glucagon infusion, whereas plasma glucose levels declined to baseline between 20 min and 3 h (Table 1). Similarly, the stimulatory effect of sustained physiological hyperglucagonaemia on hepatic glucose production in humans²⁹ and dogs²⁵ reaches a peak at 5–10 min and is totally dissipated by 30–45 min. The time course of the decrease in glucagon binding observed in response to hyperglucagonaemia in the present study, if applicable to other species, suggests that mechanisms other than altered glucagon binding account for the rapid evanescence which characterises the action of this hormone on hepatic glucose production^{25,28}.

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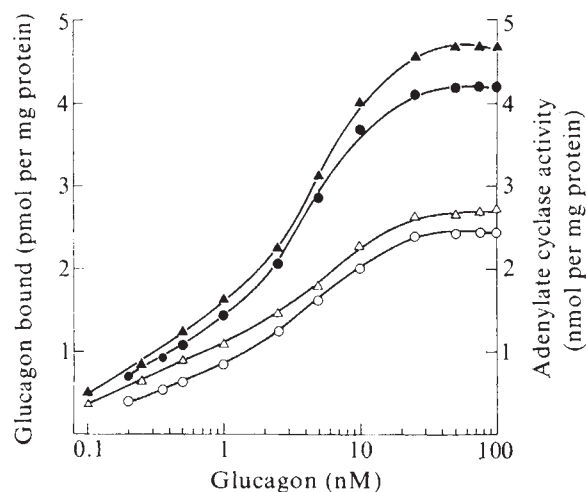


Fig. 1 Correlation between glucagon binding and glucagon-stimulated adenylate cyclase activity in liver membranes from rats infused with saline or glucagon for 5 h. In the glucagon binding studies, liver plasma membranes from saline- (●) and glucagon-infused (○) rats were incubated with 0.2 nM ^{125}I -glucagon and increasing concentrations of unlabelled glucagon for 20 min at 30 °C in glucagon binding assay conditions described previously⁸. The data are expressed as the amount of glucagon specifically bound at each hormone concentration and are plotted against the concentration of total glucagon. In the adenylate cyclase experiments, liver membranes from saline- (▲) and glucagon-infused (△) rats were incubated with the indicated concentrations of glucagon in adenylate cyclase assay conditions described previously⁸. Adenylate cyclase activity is expressed as nmol of 3', 5' cyclic AMP generated per mg protein per 10 min. The results of the binding and adenylate cyclase experiments are the mean of 12 individual experiments in each group.

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Thyrotropin-releasing hormone reversal of ethanol-induced decreases in cerebellar cGMP

THYROTROPIN-RELEASING hormone (TRH) has been shown to produce marked changes in the central nervous system unrelated to its actions on the anterior pituitary which result in the release of thyrotropin and prolactin¹. The first evidence for central effects of TRH were provided by observations that TRH potentiated L-dihydroxyphenylalanine-induced excitation in pargyline pretreated, hypophysectomised animals². Subsequently, TRH was found to antagonise the sedative and hypothermic effects of ethanol and pentobarbital³⁻⁵. These results of TRH administration may depend upon many factors, including dose, time after administration and presence of other pharmacological agents, as well as on the parameters being measured. Although the effects of TRH have been shown to be extrapituitary in nature³, biochemical and neural mechanisms that might account for its pharmacological actions have not been elucidated. Redos *et al.*⁶ recently reported that ethanol causes a dose-dependent decline in cerebellar guanosine-3',5'-monophosphate (cGMP) without significantly altering adenosine-3',5'-monophosphate (cAMP). As blood ethanol content inversely paralleled cGMP levels, they postulated that the ataxia produced by ethanol may be due to alterations in cGMP in the cerebellum. Though these changes may also reflect a feedback mechanism in response to the drug, it was of some interest to determine if these ethanol actions on cGMP were affected by TRH. We report here that decreases in cerebellar cGMP induced by ethanol are antagonised by administration of TRH and that administration of TRH itself results in significant elevation in cerebellar cGMP. These alterations in cerebellar cGMP provide the first biochemical evidence of non-hormonal actions of this tripeptide.

Male Sprague-Dawley rats (160-220 g; Zivic-Miller), after appropriate drug administration, were killed by focused microwave irradiation using a Gerling-Moore Metabostat (3.5 kW for 1.5-2.0 s). After cooling, the cerebellum was dissected from the brain and homogenised in 0.4 M perchloric acid. Following centrifugation of the homogenate for 20 min at 3,000 g, an aliquot of the supernatant was diluted with water and titrated to pH 6.2 with concentrated tris(hydroxymethyl)aminomethane base to permit direct analysis for cGMP and cAMP by the radioimmunoassay procedures of Steiner *et al.*⁷. Recovery of the nucleotides was generally 90-105%, with no cross interference noted under the assay conditions used. TRH was measured by radioimmunoassay in an aliquot of the freshly titrated extract using the procedure described by Bassiri and Utiger⁸. Protein measurements were made by the method of Lowry *et al.*⁹ and blood ethanol levels were measured by the NAD-NADH: alcohol dehydrogenase reaction (Sigma). Motor activity was measured for 10 min with Stoelting

electronic activity monitors, using maximal sensitivity settings. Student's *t*-test (two-tailed) was used to check for significant differences between groups.

In confirmation of the work of Redos *et al.*⁶, ethanol was found to produce a marked decline in cerebellar cGMP, and TRH was able to antagonise the reduction in cGMP produced by both 1.5 g per kg and 3.5 g per kg of ethanol (Table 1). Intravenous administration of 10 mg per kg TRH to animals treated with 1.5 g per kg ethanol caused the cerebellar cGMP levels to return to levels not significantly different from those in control animals. Even after 3.5 g per kg ethanol, when cGMP content was reduced to 10% of control, administration of TRH caused more than a threefold increase in cGMP, though cGMP content did not return to control levels. There was no perturbation of cerebellar cAMP content by any of the single or multiple drug administrations used, although some workers have reported¹⁰ that ethanol decreases cerebellar cAMP.

Table 1 also indicates that TRH caused a significant increase in cerebellar cGMP. A preliminary experiment (Table 1) has indicated that harmaline, an agent which causes greater increases in cerebellar cGMP than does TRH¹², will not significantly antagonise the fall in cGMP caused by 1.5 g per kg ethanol. While these data do not confirm a specific action of TRH against alcohol, it is apparent that not all compounds capable of increasing cGMP can alter the effect of ethanol on cGMP in the cerebellum.

In confirmation of earlier findings that TRH will antagonise ethanol-induced sedation, decreases in motor activity induced by 1.5 or 3.5 g per kg ethanol were found to be significantly antagonised by administration of 10 mg per kg TRH (Table 2). However, the magnitude of the TRH reversal of ethanol-induced decreases in motor activity is different from the change in cGMP induced by TRH (Table 1). The changes do, however, occur concomitantly, suggesting that they may be somehow be related. Furthermore, the peak in TRH immunoreactivity in the cerebellar supernatants (generally 150-500 ng per g tissue from control values of <10 ng per g) occurred 5 min after intravenous administration, the time at which maximal changes in cerebellar cGMP content also occurred. It should also be mentioned that animals given ethanol alone or in combination with TRH (at all doses of ethanol administered) had identical blood ethanol levels (Table 1), eliminating the

Table 1 Cerebellar cGMP after administration of ethanol, TRH or harmaline

Treatment	Cerebellar cGMP (pmol per mg protein)	N	% Control	Blood ethanol (mg dl ⁻¹)
Control	11.1 ± 1.6	17	100	< 3
1.5 g per kg ethanol	4.2 ± 1.1*	6	38	160 ± 2
TRH	20.9 ± 3.3*	19	188	< 3
1.5 g per kg ethanol + TRH	12.1 ± 1.1†	6	109	161 ± 4
Harmaline	27.1 ± 4.2*	6	244	—
1.5 g per kg ethanol + harmaline	7.7 ± 1.6‡	6	69	—
3.5 g per kg ethanol	1.2 ± 0.2*	11	10.6	> 250
3.5 g per kg ethanol + TRH	4.2 ± 1.1*†	13	38	> 250

Ethanol injected intraperitoneally as 15% (w/v) solution either 15 min (1.5 g per kg) or 30 min (3.5 g per kg) before killing; TRH 10 mg per kg injected intravenously 5 min before killing. Harmaline 2 mg per kg injected intravenously 20 min before killing.

*Significantly different from control ($P < 0.02$).

†Significantly different from equivalent ethanol dose alone ($P < 0.01$).

‡Significantly different from TRH + 1.5 g per kg ethanol.

Table 2 Effect of ethanol and/or TRH on motor activity

Ethanol dose (g per kg)	TRH dose (mg per kg)	Counts for first 10 min	% Increase caused by TRH	N
0	0	1,994.3 ± 123.7	—	6
0	10	2,692.3 ± 151.9	135*	6
1.5	0	1,315.7 ± 331.3	—	5
1.5	10	2,536.5 ± 346.3	192†	6
3.5	0	1,233.2 ± 187.8	—	6
3.5	10	3,284.6 ± 501.8	266‡	6

Ethanol given 25 min before; TRH or saline intravenously given immediately before beginning of each 10-min experiment.

*Significantly different from respective control ($P < 0.05$).

†Significantly different from respective control ($P < 0.01$).

‡Significantly different from respective control ($P < 0.001$).

possibility that changes might be related to an alteration in tissue ethanol content.

These results demonstrate marked biochemical changes in the central nervous system caused by administration of TRH alone or in combination with ethanol. The changes may be related to the behavioural antagonism of ethanol previously shown²⁻⁵, and confirmed here. The biochemical alterations induced should be useful in investigating the actions of TRH and ethanol in the CNS.

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Effects *in vitro* of the proposed antisickling agent DBA

A VARIETY of antisickling substances, both synthetic and naturally occurring, have been studied for their potential value as agents for the treatment of sickle cell disease. 3,4-dihydro-2,2-dimethyl-2H-1-benzopyran-6-butyric acid (DBA), a chemically modified derivative of xanthoxylol isolated from *Fagara xanthoxylodes*, has been reported to prevent as well as reverse sickling of sickle cell anaemia erythrocytes¹. 10 mM DBA was described as being able virtually to abolish sickling within a period of 30 min¹. We have examined the effect of DBA on washed erythrocytes prepared from blood of sickle cell anaemia patients, and report here that we have been unable to demonstrate any antisickling effect of DBA.

The methods used were as previously described². Five experiments were performed with DBA, each with erythrocytes obtained from a different patient. Oxygen equilibrium curves of the freshly prepared erythrocyte suspensions at 37 °C and

pH 7.4 showed a mean P_{O_2} at 50% saturation of 33.2 mm Hg, with a range of 29.2 to 37.0. The addition of 10 mM DBA resulted in a mean increase in the $P_{O_2}^{50}$ of 3.0 mm Hg, a change similar to that previously reported¹. The levels of intracellular 2,3-diphosphoglycerate were not affected by the addition of DBA.

The erythrocyte suspensions, incubated alone or with added DBA, were equilibrated with gas mixtures of varying P_{O_2} , and aliquots were taken for enumeration of the percentage of sickle forms. Two of these experiments, from which a similar range of values was obtained in the control studies, are shown in Fig. 1. In both of these experiments, less than 20% of sickled cells were present in fully oxygenated samples, more than 50% of the cells were sickled when the oxygen saturation was reduced to less than 70%, and at oxygen saturations of less than 50%, more than 80% of the cells were in the sickle form. The addition of DBA produced no apparent change in the relationship between oxygen saturation and the percentage of sickled cells. Similar results were obtained in two other experiments performed using the same procedure, and in one experiment in which fresh whole blood containing heparin as an anticoagulant was substituted for the washed cells.

To confirm the capability of this experimental system to demonstrate antisickling activity, we included butylurea as an additional control in some experiments; this has potent anti-sickling activity with only a minimal effect on oxygen affinity³. This compound produced a significant reduction in sickling when added in a concentration of 0.10 M. The results of the butylurea experiment shown in Fig. 1 were obtained with an aliquot of the cell suspension used in experiment 1.

The transformation of the haemoglobin S-containing erythrocyte to the sickle cell form after deoxygenation results from molecular aggregation of the haemoglobin to produce rigid, rodlike structures that distort the shape of the cell⁴. Because sickling of the erythrocyte fundamentally involves the intracellular haemoglobin, it is difficult to reconcile the findings of antisickling activity with an agent that does not enter the red cell nor bind to the haemoglobin, as has been described for

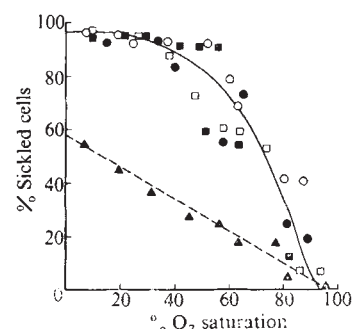


Fig. 1 Effect of DBA and butylurea on the sickling of sickle cell anaemia erythrocytes. DBA was used as supplied without further purification. A 10 mM solution of DBA was adjusted to pH 7.4 by addition of dilute NaOH, and aliquots of 15 ml were frozen and lyophilised. The DBA-sodium salt prepared in this manner was freely soluble in water as well as in the buffer used in these experiments. A solution of 0.10 M butylurea was prepared similarly. Washed erythrocytes from freshly drawn blood samples were suspended in Krebs-Henseleit buffer in a packed cell volume of 30%. A volume of 15 ml of the cell suspensions was added to each of the lyophilised compounds, and the remainder was used for the control studies. The erythrocyte suspensions were equilibrated at 37 °C with gas mixtures of varying P_{O_2} , as described previously². For each equilibrated sample the P_{O_2} , pH, and % oxygen saturation were determined, and an aliquot was collected in 5% formalin and 0.01 M Na_2HPO_4 in isotonic saline for enumeration of the % of sickled cells. Cell suspensions to which DBA or butylurea had been added were incubated with the added compound for 60–90 min before equilibration with the gas mixture. With each preparation of formalin-fixed cells, 1,000 cells were counted and classified as previously described². Results of two representative experiments are shown. Experiment 1, ■, control; □, with DBA. Experiment 2, ●, control; ○, with DBA; ▲ butylurea.

DBA. Although DBA was found to have minimal toxicity¹, the observations described here do not support the previous suggestion that this agent may be of value for the treatment of patients with sickle cell disease.

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3', 5'-cyclic AMP binds to and promotes polymerisation on platelet tubulin

In spite of considerable research, the regulatory mechanism of the reversible assembly-disassembly of microtubules remains obscure. Of the various processes and agents which have been examined as potential regulators probably none has received as much attention as cyclic nucleotides. Indeed, the number of reports which indicate that adenosine 3',5'-cyclic monophosphate (cyclic AMP) exerts a stimulatory effect in the formation of microtubules, or maintains the latter in the presence of dissociative influences is steadily growing¹⁻¹³. Most of these studies used intact cells, however, leaving unclear whether the cyclic nucleotide was directly or indirectly involved in this process. To try to resolve this problem, I have used tubulin extracted from platelets: addition of exogenous cyclic AMP has been reported to prevent cold-induced disassembly of

Fig. 1 Tubulin assembly measured by turbidimetric assay. Platelet tubulin was isolated by three cycles of temperature-dependent polymerisation-depolymerisation. Tubulin at 1.2–1.5 mg ml⁻¹ in 0.1 M piperazine-*N,N'*-bis(2-ethane sulphonic acid) (PIPES) buffer, pH 6.9 containing 2 mM MgCl₂, 4 mM EGTA and 2 mM GTP (assembly buffer), was kept at 4 °C with or without addition of cyclic AMP (dissolved in assembly buffer at 1/40 vol of platelet tubulin) for 30 min. Polymerisation was started by placing the tubulin solution into a 1-cm light-path cuvette in a temperature-controlled (37 °C) cuvette holder. The change in absorbance at 500 nm was recorded continuously for up to 20 min. *d*, Control preparation of tubulin (without cyclic AMP); *a*, 10 μM cyclic AMP; *b*, 1 μM cyclic AMP; *c*, 100 μM cyclic AMP; *e*, 1 mM cyclic AMP.

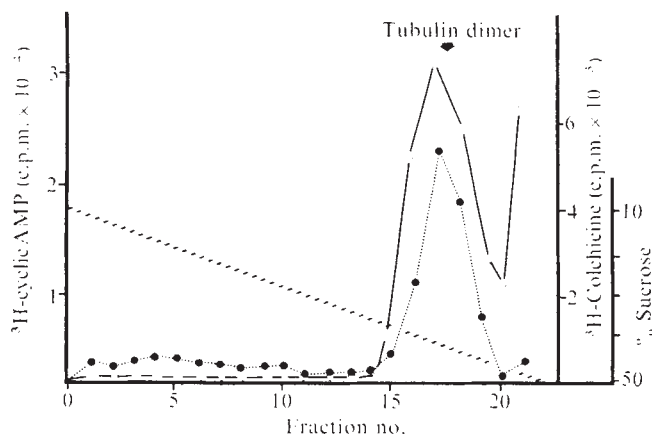
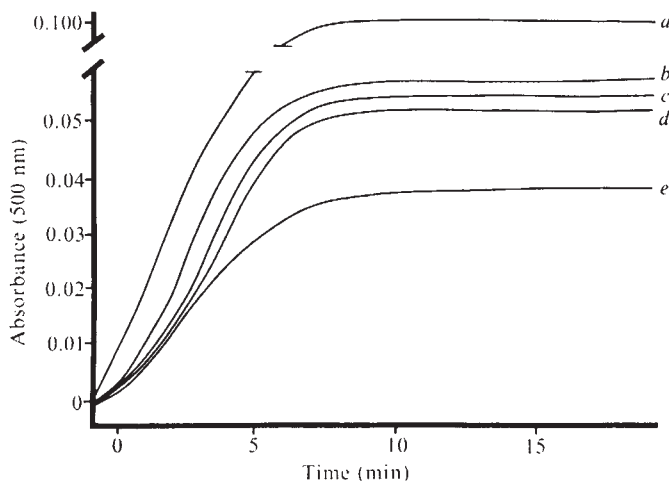


Fig. 2 Analysis of cyclic AMP binding by gradient centrifugation. 500 μg platelet tubulin (isolated as described in Fig. 1) in 0.4 ml of assembly buffer with GTP were applied, together with 4.5 nmol ³H-cyclic AMP (0.5 μCi) on to a 10–50% sucrose gradient prepared in PIPES buffer, pH 6.9 and centrifuged for 10 h at 150,000g at 4 °C. To locate the tubulin dimer, an equal amount of tubulin was preincubated for 60 min at 37 °C with ³H-colicheine (approximately 10⁶ c.p.m.) at a final concentration of 4 × 10⁻⁵ M and was then applied to an identical gradient. After centrifugation, equal fractions were collected from the bottom of each tube and radioactivities measured. ○, ³H-cyclic AMP; ●, ³H-colicheine; broken line, sucrose gradient.

microtubules in about 50% of the platelets¹⁴. I show here that cyclic AMP stimulates the assembly of microtubules and binds to platelet tubulin, primarily to the α-subunit.

Tubulin was isolated from human platelets by a previously described method¹⁵ and its purity analysed by SDS-polyacrylamide gel electrophoresis. Most experiments used tubulin which had gone through three cycles of temperature-dependent polymerisation-depolymerisation. The purity of the final tubulin preparation was greater than 98%. Polymerisation of tubulin was followed by recording the changes in optical absorption at 500 nm. Cyclic AMP in concentrations ≥ 1 × 10⁻⁶ M produced a marked enhancement of the rate of polymerisation, evidenced by a steeper slope on the absorption curve compared with control preparations of tubulin (Fig. 1). The stimulant effect of cyclic AMP was noticeable up to 1 × 10⁻⁴ M, above which it lessened markedly and finally disappeared at ≥ 1 × 10⁻³ M. The effect of cyclic AMP on microtubule polymerisation thus seems to be biphasic. This interesting finding is being investigated further and I believe that a charge effect is a possible explanation for it. Polycations are known to substitute for the microtubule-associated proteins in facilitating assembly of tubulin¹⁶, as can high Mg²⁺ concentrations¹⁷. At the pH at which microtubules were assembled in this study, most of the cyclic AMP is negatively charged. This could interfere with the attainment of an effective concentration of tubulin for microtubule formation, as tubulin itself behaves as an anionic protein.

The polymerisation of tubulin was also followed by electron microscopy. Two minutes after raising the temperature to 37 °C, a large proportion of cyclic AMP-treated tubulin had undergone assembly into microtubules, some of which were already of considerable length. In control preparations not treated with cyclic AMP, microtubules had formed only very sparingly and were relatively short, but ring structures were quite abundant.

In further experiments, I attempted to identify the site of action of cyclic AMP. High molecular weight tubulin-copurifying proteins have been described in tubulin preparations isolated from most sources. Although their exact role in initiation and elongation of microtubule formation is still an issue of some controversy, two high molecular weight proteins associated with tubulin in consistent ratio have been found to coat the surface of brain microtubules assembled *in vitro*^{18,19} and a third protein seems to have cyclic AMP-stimulated protein kinase activity²⁰. Platelet tubulin was shown to be capable of

binding cyclic AMP. Equilibrium dialysis and gradient centrifugation (Fig. 2) were used to evaluate the binding of cyclic AMP to tubulin. A Scatchard plot²¹ of the equilibrium data²¹ was linear and from this the number (n) of binding sites was estimated. In the absence of GTP, 0.6 molecules of cyclic AMP were bound per molecule of tubulin heterodimer, whereas in the presence of 1 mM GTP n was calculated as 0.9 per tubulin heterodimer (Fig. 3). The dissociation constants, K_d , determined from the data of Fig. 3 were 2.5×10^{-9} M for the former and 5×10^{-9} M for the latter. In view of the magnitude of these constants it is to be expected that cyclic AMP is bound to platelet tubulin *in vivo*. To investigate this, the amount of cyclic AMP bound to platelet tubulin was measured by radioimmunoassay²². A perchloric acid extract was prepared as described previously²³ and purified by chromatography through an anion exchange column (Ag 1 $\times$ 8, BioRad) to remove non-cyclic nucleotides, which were eluted with 0.5 M formic acid. The eluate of 2 M formic acid was analysed for cyclic AMP. Corrected for loss during extraction and purification, 1 nmol tubulin dimer was found to have 1.5 ± 0.5 pmol cyclic AMP. These results indicate that tubulin, after extraction from platelets, has no significant amount of cyclic AMP bound to it: most of the cyclic nucleotide must therefore be lost during the extraction procedure.

To try to identify the site of cyclic nucleotide ligation, a photoactive derivative of ³H-cyclic AMP was prepared²⁴. After optimal conditions for photolysis had been established, competition experiments with non-derivatised unlabelled cyclic AMP were carried out which firmly indicated that 8-azidoadenosine 3',5'-cyclic AMP (8-N₃-cyclic AMP) was fully competitive with normal cyclic AMP. Analysis of the radioactivity associated with tubulin showed that virtually all of the photoactive labelled cyclic AMP was bound to the α -subunit (Fig. 4). There was, however, another peak of radioactivity coinciding with a high molecular weight tubulin-copurifying protein. The mechanism by which cyclic AMP enhances the initiation of microtubule formation remains

Fig. 3 Scatchard plot of equilibrium dialysis data of platelet tubulin and cyclic AMP. Four experiments were carried out with platelet tubulin isolated by three cycles of temperature-dependent polymerisation-depolymerisation, while two experiments (one each with GTP and without GTP) were carried out with tubulin isolated by immunosorbent column¹⁵. Platelet tubulin at 200 μ g ml⁻¹ in assembly buffer with or without GTP was dialysed at 4 °C against different concentrations of ³H-cyclic AMP containing assembly buffer with 1 mM GTP or without GTP until equilibrium was reached. The accumulation of ³H-cyclic AMP inside the dialysis bags was measured and after correction for nonspecific absorption, the mean values of three experiments each were plotted as shown. $\blacktriangle$, Experiments with GTP; $\bullet$, experiments without GTP.

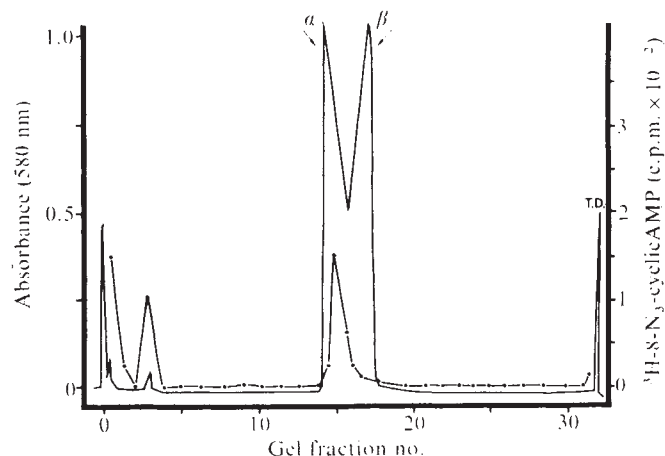
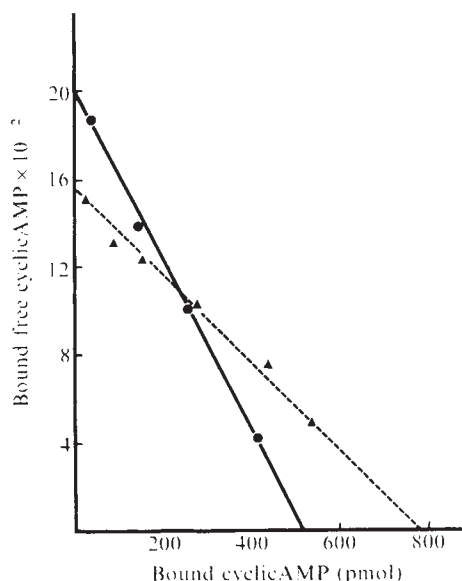


Fig. 4 SDS-urea polyacrylamide gel electrophoresis²⁵ of platelet tubulin photolysed in the presence of ³H-8-N₃-cyclic AMP. 100 μ g platelet tubulin in assembly buffer with GTP (1 mM) were photolabelled with 0.1 μ M 8-N₃-cyclic AMP, which was prepared according to Muneyama *et al.*²⁴, but with inclusion of ³H-cyclic AMP. The solutions being photolysed were placed in quartz cuvettes, which partly immersed in an ice bath, were rotated continuously while being illuminated for 60 min by a 253.4 nm UVS-11 mineral light located 8 cm from the cuvette. Tubulin was then reduced and alkylated with iodoacetamide and prepared for SDS-urea gel electrophoresis in which 0.1% was used as the reservoir buffer²⁵. After electrophoresis, the gels were stained with Coomassie blue, scanned at 580 nm, dried, sliced and counted by liquid scintillation. The anode was at the left. TD signifies the position of the tracking dye. In initial experiments, it was established that addition of cyclic AMP at approximately equimolar concentrations suppressed the binding of ³H-8-N₃-cyclic AMP by tubulin.

obscure. Based on other experiments²⁶, phosphorylation of platelet tubulin was shown to be important for polymerisation, but the protein kinase activity associated with platelet tubulin was found to be independent of cyclic AMP. Although it is easy to suggest that cyclic AMP bound to microtubule-associated high molecular weight protein can promote its stimulation of tubulin polymerisation, the significance of the ability of α -tubulin to ligate this cyclic nucleotide remains to be established.

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A single chain precursor of C4 in human serum

THE fourth component of complement (C4) in human serum is a glycoprotein with a molecular weight of 209,000 and consists of three covalently linked polypeptide chains¹⁻³. A three-chain structure in proteins is exceptional and there is a possibility that C4 is synthesised as one or two chains which are subsequently cleaved. This hypothesis is in agreement with Hall and Colten⁴, who presented evidence that polysomes from homogenates of guinea pig liver synthesised a precursor form of C4. This pro-C4 has a single polypeptide chain of the same molecular weight as intact serum C4. We

report here the presence in human serum and plasma of a functionally inactive protein with antigenic properties of human C4, which does not dissociate in reducing conditions into the α , β and γ chains characteristic of haemolytically active serum C4. This molecule probably represents a circulating precursor form of C4, or material that has escaped from the cell without being processed.

Pro-C4 was purified from human serum or plasma following the same initial steps as described for the purification of human C4 (ref. 3; for details of method, see Fig. 1). Three protein peaks could be identified in the eluates. After reduction the slowest moving protein dissociated into three chains of molecular weights 93,000, 78,000 and 33,000, characteristic of human C4. The second component migrated as a single chain of molecular weight about 200,000 and a third component (fastest moving) corresponded to a protein of molecular weight 68,000. When these proteins were analysed antigenically, the first and second peaks reacted with several antisera to human C4, whereas the third peak reacted with rabbit antisera to albumin. C4 haemolytic activity was found associated only with the protein fractions of the slowest moving peak corresponding structurally to the three subunits of human C4.

To discount the possibility that a contaminant in the anti-sera to C4 was responsible for the antigenic reactivity in the second peak, a precipitin curve was obtained using the single chain protein, and the supernatants of the reaction mixtures were assayed with purified C4 at the protein concentrations required at equivalence. The results in Fig. 2 show that the single chain protein depleted the antiserum of anti-C4 activity; therefore the single chain material contained antigenic determinants similar to those present in C4. Antigen-antibody crossed immunoelectrophoresis⁷ using anti-C4 in the second dimension and transferrin as a marker showed that pro-C4 has a more anodal mobility than C4 or C4 treated with CIs (Fig. 3).

These findings suggest that C4 is synthesised and released, at least partially, as a single polypeptide chain. The recovery of this single chain protein represents approximately 5% of the total C4 protein present in normal serum. This pro-C4, although antigenically identical to haemolytically active C4, differs from C4 in its electrophoretic mobility and the absence of functional haemolytic activity. Data supporting the presence of a single chain C4 in human plasma were recently presented by Gorski and Müller-Eberhard, who separated single chain C4 from the three chain subunits by gel filtration on Sepharose 6B in 6 M GuHCl (ref. 8).

Based on the data presented by Hall and Colten and evidence for precursor forms of other proteins^{9,10}, single

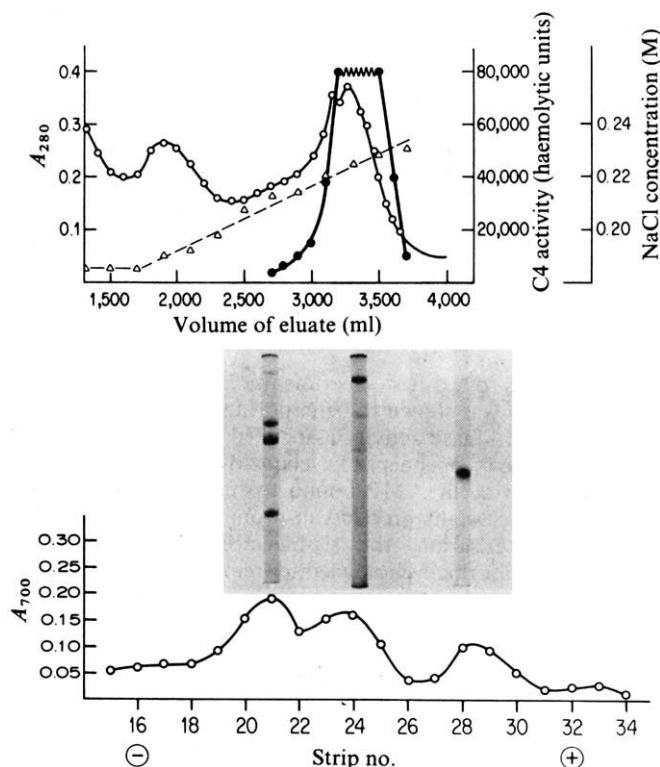
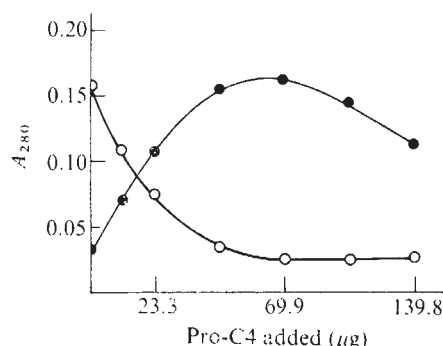


Fig. 1 *a*, Chromatography of DEAE-Sephadex A-50 column of the supernatant from the pH 5.5 euglobulin precipitation. Serum (500 ml) was diluted with 2 l of water containing 5 mM CaCl_2 , 2.5 mM phenanthroline and 2.5 mM iodoacetamide. The pH was adjusted to 7.4 with 1 M NaOH and the diluted serum was stirred at 4 °C for 2 h and centrifuged at 23,000 g for 30 min. The precipitate was separated and the supernatant adjusted to pH 5.5 with 0.5 M HCl; after stirring for 15 min the precipitate was removed by centrifugation. The supernatant was brought to pH 8.6 by adding 1 M Tris and NaCl was added to give a concentration of 0.185 M. The solution was fractionated on a column of DEAE-Sephadex A-50 equilibrated with buffers containing 0.01 M Tris HCl (pH 8.6), 0.185 M NaCl. The column was washed with the same buffer until the A_{280} of the eluate reached 0.1 and the adsorbed proteins were eluted with a linear NaCl gradient to 0.23 M. ●, Haemolytic activity of component C4; ○, A_{280} protein concentration; △, NaCl concentration. *b*, The fractions containing C4 haemolytic activity were concentrated by ultrafiltration and further purified by Pevikon C-870 block electrophoresis (30 × 45 cm) for 18 h at 450 V and 100 mA. The block was cut in half-inch strips and eluted (○, profile) with 0.01 M Tris-HCl (pH 8.6) and 0.5 M NaCl. Protein concentration was measured using the method of Lowry *et al.*⁵ and the fractions containing protein (peak samples) were analysed by 5.6% polyacrylamide gel electrophoresis in SDS. Samples were reduced⁶ fully by incubation in 40 mM dithiothreitol in SDS-urea.

Fig. 2 Precipitin curve of single-chain protein from Pevikon block electrophoresis. Goat anti-human C4 antiserum (0.1 ml) was incubated for 18 h with pro-C4 0.466 mg ml⁻¹ in a total volume of 0.4 ml. The samples were spun and the supernatant removed. After washing the precipitates were dissolved in 1 ml 0.5 M NaOH and the A_{280} measured (●). To 0.3 ml of each supernatant, 0.1 ml C4 (containing 0.700 mg ml⁻¹) was added and the protein present in the precipitate measured (○).



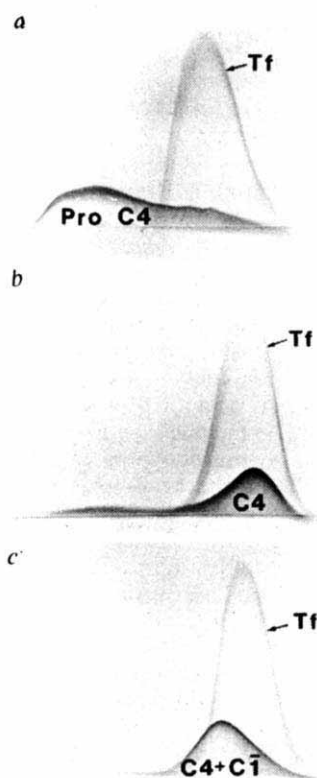


Fig. 3 Crossed antigen electrophoresis of pro-C4 (a), C4 (b) and C4 treated with purified CIs (C4b) (c).

chain C4 has been designated pro-C4. However, an alternative explanation for the presence of this molecule could be derived from the experiment of Roos *et al.*¹¹. These workers have presented evidence in the mouse of two closely related serum proteins Ss and SIp, which are immunochemically homologous to human C4. Although both of these proteins in reducing conditions yield three chains, the molecular weights differ slightly. *In vitro* synthesis of Ss and SIp has provided evidence which indicates that the molecular weight differences are controlled by the S region of the H-2 complex, suggesting that the two proteins represent two similar but structurally distinct subclasses of mouse C4 (ref. 11).

Cleavage of human pro-C4 into three fragments has been accomplished by dialysis against water followed by ultrafiltration; this degradation may be due to a trace proteinase. The individual fragments differed slightly in molecular weight from those derived from serum C4 and remained haemolytically inactive. The mechanism and sites of conversion of pro-C4 to C4 *in vivo* are not yet known.

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β' Subunit of bacterial RNA polymerase is responsible for streptolydigin resistance in *Bacillus subtilis*

BACTERIAL RNA polymerase consists of a core ($\alpha_2\beta\beta'$) and a sigma (σ) factor which form the active holoenzyme ($\alpha_2\beta\beta'\sigma$) (ref. 1). Significant differences in size of the β and β' subunits occur in the enzymes from various bacterial species². The exact functions of the larger subunits are still unknown. In *Escherichia coli* the β' subunit is the largest polypeptide and is involved in the binding of RNA polymerase to DNA³; the β subunit is responsible for both rifampicin⁴ and streptolydigin^{5,6} resistance. In contrast the largest RNA polymerase polypeptide in *Bacillus subtilis* is responsible for rifampicin resistance and has been designated as β (ref. 7). The second largest polypeptide of *B. subtilis* contains the two zinc atoms associated with RNA polymerase⁸ and has been designated as β' . We demonstrate here, using subunit reconstitution studies, that the second largest subunit of *B. subtilis* RNA polymerase—the β' subunit—is responsible for streptolydigin resistance. Since the β subunit of *E. coli* is responsible for resistance to both antibiotics, a divergence in function has occurred between the β and β' subunits of *B. subtilis* and *E. coli*. These and other studies⁹ thus show clearly that function cannot be attributed to the two larger subunits purely on their relative size. Furthermore the identification of streptolydigin resistance with the β' subunit and the map locations of rifampicin and streptolydigin resistance in *B. subtilis*¹⁰ indicate that the genes for the β and β' subunits are very closely linked and that streptolydigin resistance determines the *rpoC* locus in *B. subtilis*.

The genetic map position of rifampicin and streptolydigin resistance in *B. subtilis* has been determined^{10,11}. The two markers are very close, but have distinct and significant map distances from each other (Fig. 1). This observation suggested the possibility that streptolydigin resistance could be caused by a mutation outside of the *rpoB* locus which is responsible for rifampicin resistance.

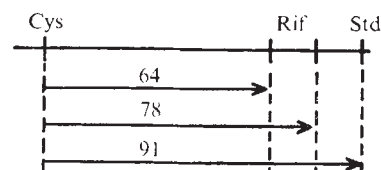


Fig. 1 The genetic map location of rifampicin and streptolydigin resistance in *B. subtilis* as determined by L. Brown¹⁰. The numbers refer to the relationship: 100 % co-transformation - map units.

In order to test this hypothesis a *B. subtilis* 168 streptolydigin resistant mutant, OSB406, was obtained from L. R. Brown¹⁰. The purified RNA polymerase from this mutant was resistant to streptolydigin at high concentrations (100 $\mu\text{g ml}^{-1}$) whereas the wild type *B. subtilis* 168 parent strain was inhibited about 70% at the same concentration of the antibiotic (Fig. 2). Pure subunits of the RNA polymerase from this mutant were combined individually with wild type subunits to determine which subunit was responsible for streptolydigin resistance of the enzyme. The

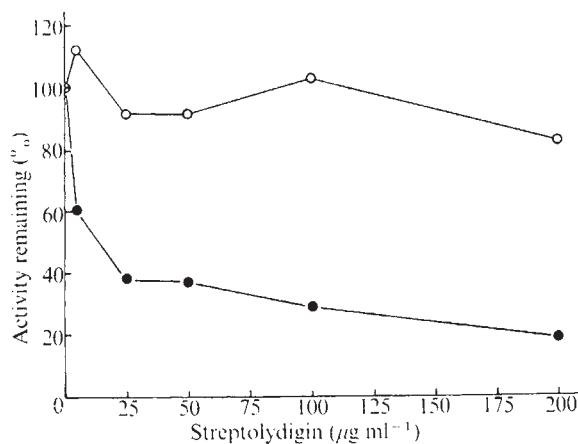


Fig. 2 Effect of streptolydigin on wild type and OSB406 (streptolydigin resistant) RNA polymerase holoenzyme activity. Each assay mixture contained 120 mM Tris-HCl, pH 7.9, 4 mM Mg acetate, 1.6 mM MnSO₄, 20 mM dithiothreitol, 0.4 mM ATP, CTP, and GTP, 0.2 mM ³H-UTP (2.87 × 10⁴ c.p.m. nmol⁻¹), 0.15 M KCl, 1 μg poly d(AT), 3 μg holoenzyme, and from 0 to 200 μg ml⁻¹ streptolydigin. Reaction mixtures were allowed to incubate at 37 °C for 10 min; then the reaction was stopped by the addition of an equal volume of 10% trichloroacetic acid containing 0.01 M sodium pyrophosphate and the mixtures were allowed to precipitate for 30 min on ice. The precipitate was collected and washed on glass fibre filters and its radioactivity was determined. Values given are in c.p.m., and each number represents the average of triplicate assays after subtraction of background counts. The percentage activity remaining after addition of streptolydigin was plotted against streptolydigin concentration. ●, Wild type; ○, OSB406.

yield of enzyme activity ranged from 6% when σ factor was absent during reconstitution to 40% when σ factor was present during reconstitution.

To identify the subunit of *B. subtilis* RNA polymerase determining resistance to streptolydigin, RNA polymerase and its subunits were purified from both *B. subtilis* 168 wild type and from OSB406 streptolydigin resistant strains by the method of Halling *et al.*⁷ (Fig. 3). This purification was aided by the development of an effective affinity column method for protease removal from *B. subtilis* extracts¹². These subunits were used to reconstitute RNA polymerase in all eight possible combinations of wild-type and mutant subunits. The reconstituted enzymes were then assayed in the presence of purified σ factor and in

Table 1 Properties of RNA Polymerase with subunits from OSB406 Std^R and wild type

Subunits	Control (c.p.m.)	+ Streptolydigin (c.p.m.)	% Inhibition (–) or stimulation (+) (c.p.m.)
α β β'	1,170	252	–78
α β β^*	1,320	2,070	+57
α β^* β'	1,547	271	–82
α^* β β'	1,466	332	–77
α β^* β^*	1,741	2,990	+72
α^* β^* β'	2,313	340	–85
α^* β β^*	1,674	2,354	+41
α^* β^* β^*	1,789	3,049	+70

Subunits were mixed to a final protein concentration of 0.2 mg ml⁻¹ as described previously⁷. Each assay mixture contained 120 mM Tris-HCl, pH 7.9, 4 mM Mg acetate, 1.6 mM MnSO₄, 20 mM dithiothreitol, 0.4 mM ATP, CTP, and GTP, 0.2 mM ³H-UTP (2.87 × 10⁴ c.p.m. nmol⁻¹), 0.15 M KCl, 2.85 μg Φe DNA, 1 μg of reconstituted core enzyme, 1.25 μg sigma factor, and 12.5 μg of streptolydigin in 5 μl of 50% ethanol, or an equivalent amount of ethanol with no streptolydigin. Assay mixtures were allowed to incubate at 37 °C for 40 min; then acid precipitable counts were determined as in Fig. 2.

*Subunits from the Std^R mutant.

the presence and absence of streptolydigin. Only those combinations of subunits including the β' subunit from the streptolydigin resistant enzyme show resistance to the drug (Table 1). Therefore, the mutation causing streptolydigin resistance is expressed in the smaller or β' subunit of *B. subtilis* RNA polymerase (Fig. 3).

Reconstruction experiments by Halling *et al.*⁷ with subunits from wild type and a rifampicin resistant mutant, Ts-1¹⁴, revealed that the core subunit responsible for rifampicin resistance in *B. subtilis* RNA polymerase was the largest (β) subunit. To confirm that the mutations in *B. subtilis* causing streptolydigin and rifampicin resistance affect different subunits, RNA polymerase was reconstituted using all four possible combinations of the β and β' subunits isolated from the RNA polymerases of the streptolydigin resistant strain OSB406 and the rifampicin resistant strain Ts-1. As may be seen in Table 2, this resulted in the formation of hybrid polymerases, resistant to either streptolydigin or rifampicin, resistant to neither drug, or resistant to both drugs (Table 2). Thus streptolydigin resistance is dependent on the presence of the β' subunit from the streptolydigin resistant strain and rifampicin resistance is again seen to be dependent on the presence of the β subunit from the rifampicin resistant strain. This is unlike the case with *E. coli* RNA polymerase in which genetic mapping and reconstitution experiments have shown that the mutations causing streptolydigin and rifampicin resistance affect the β subunit^{5,6}.

These studies have shown that *B. subtilis* mutants resistant to the streptolydigin and rifampicin are altered in the second largest core polypeptide chain (β') and the largest core polypeptide chain (β), respectively. Mutants of *E. coli* resistant to both of these antibiotics are only altered in the β subunit. Therefore relative size of RNA polymerase subunits cannot be used as a criterion for functions nor can function be ascribed to a subunit without reconstitution and genetic studies.

The second largest core polypeptide of *B. subtilis* has been designated as β' because it binds to the blue Dextran Sepharose column and contains Zn²⁺ as does the *E. coli* β' subunit (R. Wu and R. Fukuda, personal communications). The streptolydigin map site is therefore also the *rpoC* locus in *B. subtilis*. This is in contrast to *E. coli* in which the streptolydigin phenotype maps in the *rpoB* locus^{5,6}. Previous studies have implied that the β' subunit was involved primarily in the DNA binding function³; however, as streptolydigin has been shown to prevent chain elongation in *E. coli*^{15,16}, our results suggest that β' subunit may also play some part in the catalytic function of the enzyme. The presence of both Zn²⁺ atoms in the *B. subtilis* β' subunit⁷ suggests a possible role for these metal ions in the catalytic process.

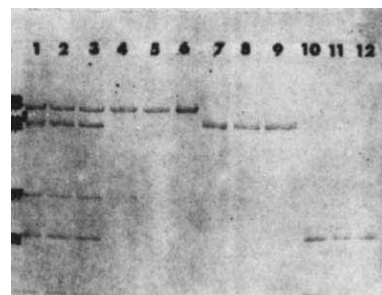


Fig. 3 Polyacrylamide gel electrophoresis of purified RNA polymerase and isolated subunits from *B. subtilis* wild type, Ts-1 (rifampicin resistant) and OSB406 (streptolydigin resistant). The SDS-urea-polyacrylamide (5%) gel was prepared according to the method of Wu and Bruening¹³ as modified by Halling *et al.*⁷. The wild-type holoenzyme and subunits were applied to wells 1, 4, 7, and 10; Ts-1 to wells 2, 5, 8, and 11; OSB406 to wells 3, 6, 9, and 12.

Table 2 Properties of RNA polymerase with subunits from OSB406 Std^R and Ts-1 Rif^R

Subunits	Control (c.p.m.)	+ Streptolydigin (c.p.m.)	+ Rifampicin (c.p.m.)
$\alpha + \beta^{\text{Std}} + \beta'^{\text{Std}}$	4,881	10,664	129
$\alpha + \beta^{\text{Std}} + \beta'^{\text{Rif}}$	6,209	1,110	86
$\alpha + \beta^{\text{Rif}} + \beta'^{\text{Std}}$	6,211	9,669	6,991
$\alpha + \beta^{\text{Rif}} + \beta'^{\text{Rif}}$	7,129	1,702	8,528

Subunits were mixed as described in Table 1, except that 0.025 mg sigma factor was added during reconstitution; assays were done as described in Table 1, except that no additional sigma factor was added, and the incubation period was for 30 min. Control assays received 5 μ l of 50% ethanol; either 12.5 μ g of streptolydigin or 1 μ g of rifampicin in 5 μ l of 50% ethanol were added to the test assays. All numbers expressed in c.p.m. are the average of triplicate assays after the subtraction of background counts. The superscripts refer to the origin of the subunits from the rifampicin (Rif) and streptolydigin (Std) resistant mutants. The α and the σ subunits used in these experiments were obtained from the streptolydigin resistant mutant.

Reconstruction of an enzyme resistant to both antibiotics showed that the mutations were not altering the conformation of the subunits severely. This result is not completely unexpected, because mutants resistant to both drugs can be isolated. However, the reconstitution event is not totally efficient even with wild-type subunits and there was an expectation of lowered reconstitution efficiency with mutant subunits. An interesting aspect of our results is the 'stimulation' of the reconstituted drug-resistant enzymes by the presence of the antibiotic in the reaction mixture. The stimulatory effect was observed consistently in our studies and is not due to the ethanol in which the drug is dissolved. These results suggest that the antibiotics may bind weakly to the reconstituted enzymes and not inhibit its activity, but perhaps 'improve' the conformation of the enzyme and therefore make it more active.

The *B. subtilis* and *E. coli* RNA polymerase cores differ in two significant ways. The β and β' subunits in *B. subtilis* are smaller in molecular weight than those of *E. coli*⁷ and the functions of the β and β' subunits differ with respect to their binding of rifampicin and streptolydigin. This clear difference in structure and function of the subunits of two prokaryotic RNA polymerase indicates that evolutionary divergence has occurred in the transcriptional machinery. This divergence could be the result of a normal variability permissible for an enzyme function. However, the increasing complexity of *B. subtilis* RNA polymerase revealed by recent studies¹⁷⁻²², may reflect the necessity for a higher degree of enzyme evolution for an organism which undergoes transcriptional regulation during differentiation²³.

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Solvent-depleted bilayer membranes from concentrated lipid solutions

MODEL bimolecular lipid membranes (BLMs) contain lipid solvents, such as *n*-alkanes, which are not normally present in biological membranes^{1,2}. Studies aimed at overcoming this limitation have established that the volume of solvent within the BLM can be estimated by measurement of the bilayer specific geometric capacitance³⁻⁶. We have used this approach to evaluate the distribution of solvent between the BLM and its supporting organic bulk solution (torus). Decreasing the solvent content of the torus solution resulted in a decrease in the solvent content of the BLM. Moreover, membranes that are nearly free of residual solvent could be formed from highly concentrated lipid solutions having a minimal amount of solvent. The observed concentration dependence of BLM capacitance may reconcile the differences between membranes formed from submerged bulk solutions and those formed from excess lipid monolayers⁷.

BLMs were formed at room temperature (22±0.5 °C) by our conventional technique⁸ in which an air bubble is used to paint a submerged lipid/alkane solution over an aperture (1.3 mm²) separating electrolyte (1 M NaCl) filled compartments of a Teflon chamber. BLMs were formed from solutions of α -monolein (99+ % grade, Sigma) in spectral grade (99+ %) *n*-decane or *n*-hexane (Aldrich). Membrane capacitances were measured at 1 kHz (±50 mV) with a precision (1%) a.c. impedance bridge adapted from the design of White⁹. The specific capacitance of the flat circular bilayer was calculated from membrane area measurements made with a calibrated 50× microscope.

The specific capacitances (C_s) were determined for bilayer membranes formed from lipid solutions encompassing a wide range of concentrations. A significant increase of the membrane specific capacitance was observed on increasing the lipid concentration in the forming alkane solutions. Apparently, this concentration dependence of C_s has not been previously observed as earlier workers studied very dilute organic solutions near the critical micell concentration (1.75 mM for decane) above which the lipid content of the BLM is expected to remain constant¹¹. The relationship between C_s and lipid molarity (M) was found to be linear. Thus, for bilayers formed from monolein dissolved in decane, C_s values are well described by the regression line drawn to the equation $C_s = 0.18 M + 0.399$ with a correlation coefficient $r = 0.99$ for the full range of lipid concentrations ($M = 0.01$ to 1.5) (Fig. 1). The low concentration intercept ($C_s^0 = 0.399 \mu\text{F cm}^{-2}$) is expected and found to be close to C_s values reported by others^{6,7,12,13} for M near 0.01. The increase of C_s with lipid molarity implies that bilayers formed from concentrated lipid solutions are thinner and, therefore, contain less solvent. This leads us to propose that the solvent content of the bilayer is primarily determined by the solvent content of the membrane forming solution.

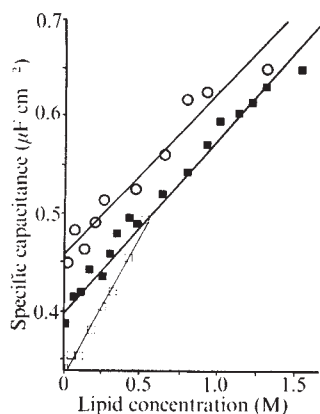


Fig. 1 Concentration dependence of lipid bilayer capacitance. The linear increase in membrane specific capacitance (C_s) with increasing membrane forming solution lipid molarity is attributed to a reduction of the amount of residual solvent within the thin bilayer. The rate of change of C_s is nearly the same for bilayers formed from monoolein/decanoic acid (■) and monoolein/hexane (○) solutions but it is greater for membranes made from lecithin/decanoic acid (□) solutions. Bilayer dielectric thickness d (nm) could be calculated from $d = 8.85 \times 10^{-7} D/C_s$ with $D = 2.14$.

The concentration dependence of C_s seems to explain the thickness differences that have been consistently observed for BLMs formed by the Mueller and Rudin bulk solution technique¹ and the Montal and Mueller monolayer technique⁸. For example, at a lipid concentration of 1.5 M, which is near the upper concentration limit for membrane formation, we observe a capacitance of $0.649 \mu\text{F cm}^{-2}$ (± 0.019 s.d.) for BLMs made from monoolein in decanoic acid. This large C_s value exceeds that reported for nearly solvent free bilayers from dilute monoolein/hexadecane bulk solutions ($0.62 \mu\text{F cm}^{-2}$) (ref. 4) and approaches the value reported for BLMs formed from monoolein/hexane monolayers ($0.735 \mu\text{F cm}^{-2}$) (ref. 7). We have considered the possible influence of the solvent species in order to explain the small capacitance difference between BLMs formed from concentrated lipid solutions and from monolayers. Figure 1 shows the observed concentration dependence of the capacitance for monoolein BLMs made from the hexane containing solutions. The C_s - M relationship for this lipid-solvent system is well described ($r = 0.97$) by a linear regression line drawn to the equation $C_s = 0.17 M + 0.446$. Indeed, the overall dielectric behaviour of the bilayers from hexane solutions is very similar to that of bilayers from the decanoic solutions. The primary effect of using hexane rather than decanoic acid as a lipid solvent is a displacement of C_s to larger values corresponding to a 0.7 nm decrease in the bilayer hydrocarbon thickness at equal lipid concentrations. The largest value of C_s obtained for BLMs from the monoolein/hexane bulk solution was $0.666 \mu\text{F cm}^{-2}$ at a concentration of 1.3 M, beyond which BLMs could not be formed. This C_s value is slightly less than that reported for so-called solventless bilayers made from monolayers. The similarity of the maximal C_s values for membranes made from concentrated lipid bulk solutions and from solvent depleted monolayers suggests that both techniques are nearly equivalent in yielding BLMs which are virtually solvent free.

We have explored the generality of our observation of concentration dependent bilayer capacitances by measuring C_s values of membranes formed from a heterogeneous biological phospholipid (lecithin, Sigma type II s—a phospholipid extract of soy beans). Bilayers of this lipid exhibited C_s values that increase with the lipid concentration of the forming solutions. Furthermore, as shown in Fig. 1, this increase is well described ($r = 0.98$) by the regression equation $C_s = 0.30 M + 0.331$ (molecular weight 800; density, 1). Compared with monoolein bilayers, the phospholipid bilayers have smaller

C_s values which increase more steeply with lipid concentration. Nonetheless, the overall features of the concentration dependence of C_s are essentially the same. It seems that for a variety of lipids, it is possible to deplete the solvent content of bilayers by using highly concentrated (that is, alkane depleted) bulk solutions. The concentration dependence of dielectric thickness is expected to be useful not only for providing BLMs which continuously encompass a wide range of thicknesses, but also for forming nearly solventless BLMs using the conventional bulk solution technique.

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Errata

In the letter 'Apomixis may be widespread among trees of the climax rain forest' by A. Kaur *et al.* *Nature* **271**, 440–442, in paragraph 2 line 12, for 'tiploidy' read 'triploidy'. In paragraph 6 line 19 should read: *macroptera* it is, and it is

In the article 'Silicalite, a new hydrophobic crystalline silica molecular sieve' by E. M. Flanigan *et al.* *Nature* **271**, 512–516, in paragraph 5 line 3 for 2 d read 2 h; in line 5 for $0k1.k+1$ read $0k1, k+1$. In paragraph 6 line 11 should read . . . has the same topology as that reported for 'shape-selective' . . . In Fig. 1 legend for c read b .

In the letter 'Estimate of the volatile nitrosamine content of UK food' by T. A. Gough *et al.* *Nature* **272**, 161–163 the abscissa in Fig. 1c should read 0.01, 0.1 and 1.0 and not 0.01, 0.1 and 10.

Corrigendum

In the letter 'The neural representation of visual space' by N. Drasdo, *Nature* **266**, 554; in paragraph 1 line 11 for $\sqrt{D_0}$ read D_0 . In Fig. 1 displace the symbol □ at 10° on nasal meridian 2.4 mm upwards. On page 555 paragraph 3 line 13 for 15.6 read 15.1.